Quarterly Review of MEDICINE

VOLUME 9 NO. 3 **AUGUST 1952**

Incorporating the International Record of Medicine

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FOREWORD

The Quarterly Review of Medicine is devoted to the presentation of selected abstracts encompassing the various subdivisions and related fields of internal medicine. Not only the content but the status of the material in its relationship to present-day knowledge and concept influences the basis of selection. Special review articles and advanced experimental and clinical reports on the subjects of current interest are also included. It is hoped that noteworthy trends in the field of internal medicine will thus be recorded and a proper perspective on the rapidly changing and dynamic state of medical knowledge attained.

The abstracts and editorial comments are grouped together under the following headings:

- INFECTIOUS DISEASES
- ONCOLOGY
- RESPIRATORY DISEASES
- CARDIOVASCULAR DISEASES
- GASTROINTESTINAL DISEASES
- GENITOURINARY DISEASES
- MUSCULOSKELETAL DISEASES
- NEUROLOGY AND PSYCHIATRY
- HEMATOPOIETIC DISEASES
- ALLERGY
- METABOLIC AND ENDOCRINE DISORDERS
- GERIATRICS
- DERMATOLOGY AND SYPHILOLOGY
- DISEASES OF DOUBTFUL ORIGIN
- BASIS SCIENCES
- INTEGRATION WITH ALLIED SPECIALTIES

BOOK REVIEWS

NEWS, NOTES AND COMMENTS

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Newer Methods of Blood Collection

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The purpose of a technical procedure is often lost in the hysterical joy of inventing equipment and elaborating technic. This is particularly true of a procedure that has challenged man's creative imagination for four centuries.

In 1840, Richard Oliver penned a classic description of the purpose of today's symposium.[1]

"Blanched by profuse hemorrhage, which no adequate means had been employed to suppress, but which had now ceased, she was lying on her back in a state of imperfect consciousness, with the pulse at her wrist barely perceptible . . . deceitful promises of reaction were succeeded by progressive indications of sinking . . . I was provided with the apparatus necessary for performing transfusion; and having obtained a willing supply of blood from three of the patient's kind-hearted neighbors, I opened a vein at the bend of the elbow . . . we had the very perfect gratification of witnessing not only the complete restoration of the circulatory powers, but the return of consciousness."

It is obvious that Oliver transfused enough fresh blood to substitute for that lost by profuse hemorrhage (figure 1). It is paradoxical that a century of progress in technic has improved the availability and quantity rather than the quality of blood used for substitution therapy.

The fresh blood transfused by Oliver differed markedly from the blood infused into almost all patients today. Current knowledge tells us that the cells in his blood were a living population with normal life expectancy. All old, degenerated cells had been scavenged by the donor's reticuloendothelial system. The erythrocytes, platelets, and leukocytes present in the blood retained functional capacity and effectively restored the red cell mass and bolstered the clotting mechanism.

Given at a Symposium on "Frontiers of Research on Blood and Plasma Extenders," marking the formal dedication of the new Sharp & Dohme Medical Research Laboratories, West Point, Pennsylvania, May 12, 1952.

For the convenience of our readers we are continuing the consecutive paging of the *Quarterly Review of Medicine* Volume 9, as prior to its incorporation in *International Record of Medicine.*

FIG. 1. An early method of blood transfusion.

About one per cent of the donor's cells died of old age daily and were scavenged. The youngest of the infused cells disappeared from the recipient's circulation 100-120 days after transfusion (figure 2).[2]

Contrast this with blood supplied by modern blood bank technic. Senescence of red cells proceeds in the refrigerator at the same rate as *in vivo*. As the population ages, an increasing percentage of red cells irreversibly deteriorates. When the blood is infused, both viable cells and the superannuated cells find their way into the patient's circulation. The latter are removed from the circulation rapidly, even during infusion (figure 3).[2]

The freed hemoglobin may be a distinct liability to the patient with peripheral vaso constriction and renal ischemia.[3] To others, the deficit in viable red cells may cause five infusions to be given to accomplish the repair usually expected after three transfusions of fresh blood.

Blood degradates in other ways too complex for consideration here. The able clinician will, therefore, choose his blood collecting technic to fit his individual patient's need rather than to suit the convenience of a blood program. Four kinds of blood are available to satisfy distinct clinical needs.

The first of these is whole blood taken from the donor and infused into the patient with minimal delay. Modern technic of blood collection removes the factor of common

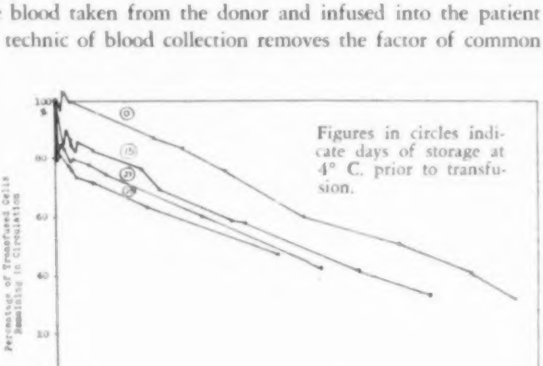
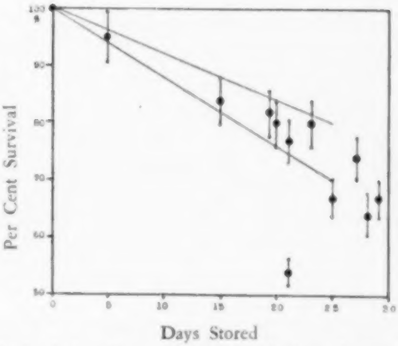


FIG. 2. Curves of disappearance of transfusion cells in four typical experiments, as determined by the Ashby technic. There is an immediate removal (48 to 72 hrs.) from circulation of red cells which have undergone irreversible changes during storage. The remaining cells disappear at a rate closely approximating that of normal cells and retain unimpaired functional capacity. The safety of transfusion of stored blood is limited by the amount of red cell destruction during the immediate post-transfusion period. When the immediate loss of cells exceeds 30 per cent the blood is unsafe for therapeutic use.

Fig. 3. The post-transfusion survival of human erythrocytes collected, stored at 4° C. and transfused in plastic equipment (Fenwal Blood Pack and Recipient Set) as whole ACD blood. The percentage survival of erythrocytes of 12 transfusions are plotted, showing the limit of error (±5%) of the Ashby technic as used. The area enclosed by slopes drawn through the upper and lower limit brackets represents the performance characteristics of whole ACD blood in plastic equipment.



site and time of collection and infusion typical of the direct blood transfusion. A good hemo-repellent system permits the blood to be collected through a suitable phlebotomy needle into plastic bags for subsequent infusion without the use of anticoagulant.[4] Periods up to six hours have elapsed between collection and infusion without clotting. This type of blood has proven useful in hemorrhage during obstetrical procedures, thrombocytopenia, and following the hemorrhagic type of transfusion reaction. A donation of blood from a polycythemic donor is often spectacular in the treatment of the latter problems.

The second type of blood is decalcified blood collected through a cation exchange column.[5] It has proven particularly useful as a starting point for plasma fractionation, for loading artificial heart-lung apparatus, in many hemorrhagic problems, and in cases where low sodium and potassium content are important as in certain postoperative states

Fig. 4. The equipment for the ACD technic consists of a laminar flow phlebotomy needle, an integral donor tube, and a collapsible bag of polyvinyl resin so designed that the unit is sterilized, assembled and filled, ready for use, by exposure to saturated steam at 121 C. for 30 minutes.

and nephrosis. When collected into dextrose, the storage period for effective viability is four to five days.

The third type of blood is collected in the commonly used ACD anticoagulant and nutrient solution. It should be used as fresh as possible, although its infusion is safe and effective for three quarters of the transfusion problems up to 25 days and under emergency conditions for 30 days. Remembering always that as the medical resources of a community are diluted through paucity of supplies or overwhelming casualties, ideal therapy also is abandoned in the effort to control mass morbidity and mortality. Old blood should be avoided in civilian practice, particularly in severe shock, burns, crush injuries, and when multiple infusions are contemplated because the free hemoglobin may damage ischemic renal tissue.

The fourth type of blood consists of resuspended red cells. This permits maximal additions to the patient's red cell mass with but half the fluid volume, minimal increase in oncotic power, and elimination of 75 per cent of the sodium content of whole blood. Patients with low cardiac reserve, chronic anemia, and the nephritides benefit from this technic.

At this point a warning is indicated. There is a definite risk involved with every transfusion. Hemolytic reactions still occur because of errors in labeling, confusion of pilot tubes, infusion of the wrong bottle of blood, and lack of observation of patients during infusion. Patients sensitized to subgroups and rarer blood groups by previous transfusions must be watched for. Infected blood causes death just as spectacularly as transfusion may save life. Homologous serum hepatitis is an undectectable hazard. The mortality rate for transfusion is higher than that of appendicitis or of ether anesthesia. Accordingly, there must be a positive indication for every transfusion.[6]

Plastic equipment has been designed to provide the types of blood indicated by clinical need. It also provides maximum protection for the donor. It ensures maintenance of asepsis and safe storage. Fool-proof identification of pilot aliquots for typing, grouping, and crossmatching is designed into the technic. There are many logistic advantages in the use of this equipment. The new equipment increases the stability of the blood and makes infusion more effective.

Syncope with its apprehension, perspiration, nausea, and often prolonged weakness is a major deterrent to many donors. Slow withdrawal of blood permits compensation for the change in blood volume due to phlebotomy and donor reactions are minimal. The fact that the plastic bladder contains only liquid is positive assurance that air embolism, a real hazard recognized by experienced phlebotomists, can not occur.

The equipment for the ACD technic consists of a laminar flow phlebotomy needle, an integral donor tube, and a collapsible bag of polyvinyl resin so designed that the unit is sterilized, assembled and filled, ready for use, by exposure to saturated steam at 121 C. for 30 minutes (figure 4).

Phlebotomy is accomplished with a specially ground and polished stainless steel cannula that flares to meet the wall of the donor tube to preserve laminar flow and thus prevent platelet aggregation. An adherent, hemo-repellent film of tris (2 hydroxyethyl) dodecyl amine* is applied to the needle to delay the initiation of clot formation.[7] Blood

* The Armour Laboratories, Chicago.

will flow continuously for over two hours impelled by venous pressure alone at the rate of 17 cc. per minute through such a needle lying in an antecubital vein.

The polyvinyl resin is chemically inert to biologic fluids and is nonirritating to tissue. Its glossy surface is hemo-repellent. Tubing made of it is elastic and flexible enough so that a needle lying in a vein will determine the position of the tubing even though there is limited motion of the vein. The plastic is clear, colorless and transparent so that the provision of viewing chambers is unnecessary. A film of this plastic can be heat sealed. It is a bacterial barrier. It transmits water vapor but is not an osmotic membrane.

The blood or those fractions separable by gravity are removed from the bag through suitable delivery tubes that are closed by a diaphragm that forms a barrier across the tubes. Outer ends of the tubes are enclosed in a pouch to prevent bacterial contamination.

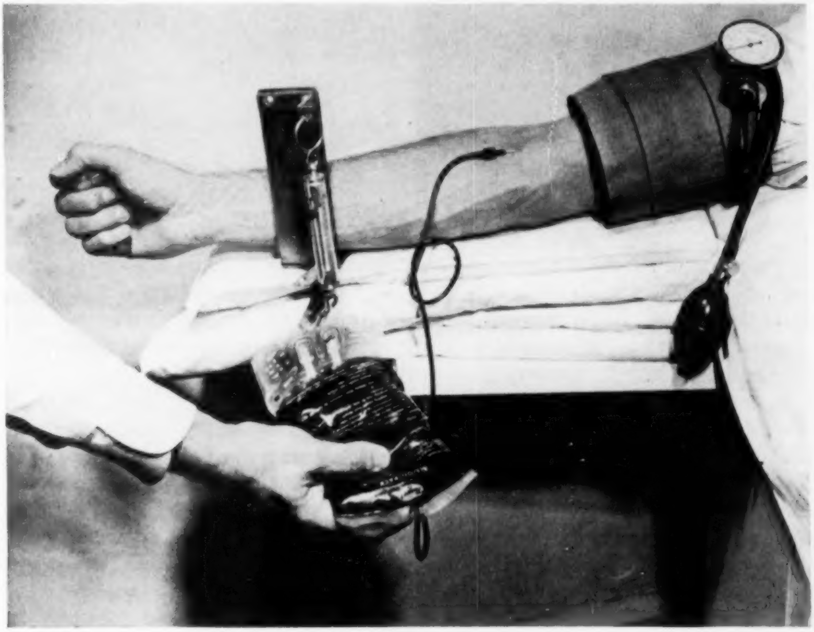


Fig. 5

The technic for using the bags is simple: the donor lies supine with a pneumatic tourniquet distended to 60 mm. Hg. applied to the arm (figure 5). The skin overlying the antecubital fossa is disinfected. A wheal of one per cent procaine hydrochloride is raised in the skin and the tissues about the vein are infiltrated. A loose overhand knot is formed in the donor tube just distal to the needle, and the bag is suspended on a spring scale with the delivery tubes uppermost. The protective sheath is removed from the phlebotomy needle. Phlebotomy is accomplished. When 640 Gm. have been collected, the knot is set tightly to seal the bag. The pressure is then released from the tourniquet and the needle is withdrawn from the vein.

FIG. 6

FIG. 7

The blood in the donor tube is sealed off in aliquots by applying R-F energy across the tubing while it is compressed in the jaws of a sealer (figure 6). The final seal is made 2 cm. distal to the junction of the donor tube with the bag so that this short segment can be sealed off after 48 hours quarantined storage and submitted for bacterial culture.

Samples of citrated blood can be preserved for crossmatching by stripping the blood from the donor tube and permitting it to refill with citrated blood prior to sealing off the tube at the bag. This is done by flexing the tube over the edge of a throat stick just proximal to the knot and drawing the stick to the bag, leaving collapsed tubing distal. Serum for serology and grouping can be obtained by sealing off whole blood in the end of the tube prior to stripping the blood from the remainder. Segments of the donor tube are cut free and centrifuged in a 15 cc. trunnion cup filled with water. An aluminum tubing clip is applied at the plasma-red cell interface, and the seals are cut from either end. Individual drops of plasma or red cells can be ejected by slight pressure on the appropriate tube.

The hermetically sealed bag is suspended from a metal stand. The pilot tubes may be inserted in the indentification tag fastened to the bag. The unit is refrigerated at 4 C., plus or minus one degree.

The blood is administered by pulling apart the tabs of the protective pouch about the blood delivery tube. The coupler of the recipient set is twisted into the lumen of the delivery tube simultaneously, to penetrate the diaphragm and couple with the delivery tube (figure 7).

Positive pressure infusion is readily performed by slipping the bag, with a recipient set attached, into a pneumatic press where controlled pressure is applied to force the

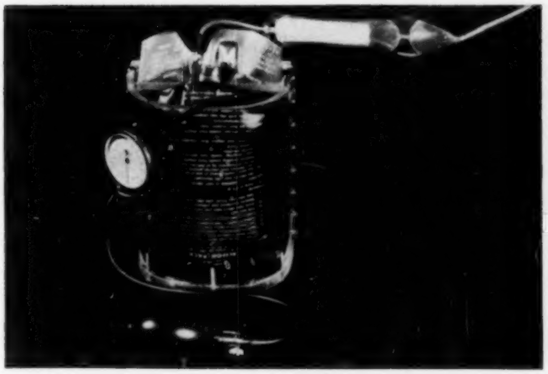


FIG. 8. Positive pressure infusion is readily performed by slipping the bag, with a recipient set attached, into a pneumatic press where controlled pressure is applied to force the blood out of the bag.

blood out of the bag (figure 8). Appropriate pressure is applied to attain the desired flow.

Plasma can be removed from the bags following either sedimentation or centrifugation. For the latter, the bag is placed in the conventional 600 cc. trunnion cup, and the space about the bag is filled with water. After properly balancing the opposed cups, the cells are packed by 20 minutes exposure to 2100 x G. centrifugal force. The bag is removed from the centrifuge and placed in the pneumatic press described above. The plasma delivery tube is exposed by pulling apart the protective pouch, and the diaphragm is pierced by a coupler connected by tubing to the plasma-pooling bottle. Air is pumped into the press to force the plasma out of the bag. As the plasma-red cell interface rises into the delivery tube, a clamp is applied to effect accurate separation. By this technic, over 99 per cent of the plasma can be removed, an eight per cent increase over older methods. The cells can be resuspended or washed by using the plasma delivery tube as the port for adding the appropriate solution. When resuspension is completed, the delivery tube is resealed by clamping it in the jaws of the R-F sealer and applying current for five seconds. The resuspended red cells are infused by the technic described for the administration of blood.

Logistic advantages of this equipment are many. The bag of ACD with its integral donor set weighs 115 Gm. packaged in an aluminum tube—16 per cent of the weight of a conventional blood container. The integral donor set obviates a separate part to be procured, inventoried, and distributed. The equipment can be shipped or stored in temperatures ranging from —56 to plus 76C. As packed commercially, four bags sealed in a tin can weigh 586 Gm. and occupy 1100 cc.—13 per cent of the space occupied by conventional equipment with comparable function. The bags weigh 620 Gm. when full of blood in contrast to 1070 Gm. for a full bottle. Two bags of blood can be shipped in the space occupied by one bottle. The cost of transporting plastic equipment charged with ACD to the donor center and returning the blood to the point of origin is 25 per cent of that for glass.

The progenitor of this plastic equipment was first illustrated in Ettmuller's "Nouvelle Chirurgie" in 1691.[8] A goose quill, treated with oil from the preen gland, tied in the neck of an inverted pig bladder served early transfusionists well. Modern technicology applies Ettmuller's functional design to the care of patients in a standardized form.

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Preservation of Whole Blood and Red Cells

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In a majority of instances where transfusion is needed, the agent of first choice is compatible whole blood. Even when whole blood is not the most desirable agent, resuspended erythrocytes are often indicated. The development of modern blood banking technic, with its extensive understanding of blood typing, has made both whole blood and resuspended erythrocytes available in a manner not believed possible a few years ago. There remains, however, a single major obstacle to the fullest realization of the potentialities of these materials. That obstacle is the inability to maintain blood in a state suitable for transfusion for periods longer than about 21 days.

This is not for lack of effort to develop methods that will provide for prolonged blood storage. But, though evidence of some small progress in this field will be presented, there are as yet no really satisfactory practical technics for a major extension of the life of blood outside of the organism. So much effort has been spent on these studies in so many laboratories that it seems desirable at this time not only to report the present status of blood storage but also to re-examine the nature of the problem in the hope that such an examination may suggest the path to new research.

Let us first examine the problem. Blood is an extremely complex tissue which, like other tissues, exists in the body in a dynamic state. We know that it consists of three major types of cellular elements—erythrocytes, leukocytes and platelets and a fluid portion, plasma — in which some 35 proteins in solution have been identified. Now, plasma is known to be useful for most transfusion purposes long after it has lost many of the properties characteristic of its native state. Platelets and white cells, even when collected by the most careful methods, are probably destroyed soon after transfusion, but this destruction causes no major difficulty. These cellular elements, while vital in special instances, are not essential in most transfusions. In contrast, red cells provide the increased oxygen transport that is most often needed in transfusions, and the rapid destruction of defunct red cells in the body causes severe clinical reactions. Thus, superficially at least, the problem of blood storage consists in keeping the predominant cellular element—the erythrocyte—in a state in which it can maintain its major function of oxygen transport.

It was stated, however, that blood exists in a dynamic state in which there are many interactions among the components. Our knowledge of these interactions insofar as erythrocytes are concerned is not yet extensive, but it is suggestive. An antisphering factor present in plasma helps preserve the biconcave discoidal shape of the erythrocyte.[1]

Given at a Symposium on "Frontiers of Research on Blood and Plasma Extenders," marking the formal dedication of the new Sharp & Dohme Medical Research Laboratories, West Point, Pennsylvania, May 12, 1952.

It has been reported[2] that tagged cholesterol introduced into the blood stream is taken up rapidly by the beta-1 lipoprotein of plasma, and that this cholesterol exchanges rapidly with the cholesterol of the red cell membrane. Preliminary data from the laboratory of Tullis[3] suggests that a nucleoprotein released by white cells will hasten the destruction of red cell membranes. In our laboratory it has been shown that a protein from plasma will lend increased stability to the red cell membrane. The well-known interaction of isohemagglutinins with red cells has given rise to the extensive field of blood typing. I am suggesting that maintenance of erythrocytes in whole blood can only superficially be considered a problem to be solved by attention to the red cell alone. In the final analysis, it is unalterably interrelated with the maintenance of the other components of blood because of these, and probably of other yet unrecognized, natural interactions. It is perhaps for these reasons that the problem of storing red cells separated from plasma components has appeared somewhat more amenable to manipulation than has the storage of whole blood.

Even though we may not legitimately concern ourselves with erythrocytes alone, it is the state of the erythrocyte that ultimately defines the suitability of blood for transfusion. Laboratory data have so frequently been misleading in forecasting *in vivo* survival of red cells that there is a tendency, where possible, to dispense with such data in blood storage studies and to go directly to the clinic. The clinic must always provide the final answers to medical problems; but in this instance, the number of variables to be manipulated is so great that it would appear to be absolutely essential to do preliminary laboratory studies for selection of those technics most likely to justify clinical study, provided, of course, such laboratory studies can be shown to have meaning.

Some of the confusion in the determination of the status of red cells during storage may have arisen from what appear to be two avenues of approach to the problem—nourishment of the cell and maintenance of structural integrity. Although their metabolic activity is atypical, mature mammalian erythrocytes, nevertheless, maintain an active glycolytic process and are equipped with an additional incompletely understood oxidative mechanism. These mechanisms provide energy for the maintenance of hemoglobin in an active state and for the transport of substances into and out of the cell. A continuing supply of dextrose, or other nutrient, is necessary to both mechanisms. There is evidence that when this nutrient is completely lacking to active cells, the cells can never again be made useful.[4, 5] In other words, the cells must be fed during storage. The erythrocyte contains a structural frame necessary, among other things, for the prevention of the escape of the tightly packed intracellular hemoglobin into the plasma. This structure is a selectively permeable network of protein, lipide, and polysaccharide, several of its proteins being enzymes. Current concepts suggest that transport through this structure to the interior of the cell is dependent on energy derived from the cell's metabolic processes and is accomplished by specific carriers.[6, 7] When this transport ceases, the cell structure may disintegrate.[8] While maintenance of the metabolism of the cell and maintenance of its structure are actually inseparable processes, they sometimes appear to be separable because the measurements used for each are specific. Those used to determine structural integrity may give little information on the metabolic state of the cell, and the reverse is equally true. Many studies of erythrocyte-aging have employed only

one type of measurement and have thus often yielded data of no meaning for the potential life of the cell after transfusion. To take extreme examples, application of fragility measurements to cells treated with formaldehyde or acrolein will indicate great stability of the structure of such cells. The selective permeability of these cells is lost, however, and the pigment is converted entirely to an inactive and useless form. Again, certain types of blood collection provide erythrocytes of unusually high glycolytic activity, suggesting cells of unusual vitality; yet in the presence of this heightened activity, the cell membrane is exceedingly fragile.

Like many other investigators, we have attempted to circumvent some of these difficulties by using routine measurements touching divergent cell propetries. Although it is not suggested that these will be found to be the technics of ultimate choice, we presently employ osmotic fragility studies of cells previously equilibrated with plasma as an indication of structural integrity. The determination of cholinesterase, an enzyme located in or on the cell membrane, serves as an indication of the activity of the membrane. And Warburg manometric measurement of the anaerobic glycolytic potential of washed cells suspended in Krebs-Ringer bicarbonate solution gives indication of the metabolic state of the cell. Until very recently this last measurement had been misinterpreted. We had assumed that it was the absolute amount of glycolysis per cell that was important. On this basis, however, the data did not correlate with clinical observations recently made available to us from the laboratories of Dr. Clement Finch of the University of Washington, Dr. Max Strumia of Bryn Mawr Hospital, and Dr. Charles Graham of Washington University. If, however, glycolytic activity is calculated as the per cent of the original glycolytic activity of the particular cells in question, the data, when correlated with the other two measurements, seem to forecast, with some accuracy, the probable percentage survival of transfused erythrocytes. We often find these three measurements yielding divergent indications of the state of the cells being examined. Obviously, only studies in which the three types of measurement correlate can be considered to be hopeful.

The problem of feeding the cell during storage obviously can be abetted by slowing the rate of metabolism and reducing the demand for nutrient by reducing the temperature of storage. This approach has been carried by several investigators to the point of freezing the blood. When one freezes, however, the structural portion of the cell may be disrupted by the transition to and from the crystalline state. This problem has been met by Strumia by freezing at —3 C.,[9] by Sloviter by immersing the cell in glycerine prior to freezing,[10] and by Luyet by vitrification.[11] In all cooling, however, a problem is presented in that the integrated enzyme systems of metabolism are slowed at different rates, producing a slowly increasing imbalance in intermediate metabolites,[12] and resulting in what Finch has recently called a biochemical lesion. This lesion is partially reversible *in vitro* by equilibration of the cells with fresh plasma and, up to a certain point, completely reversible *in vivo*.[13] But accumulation of metabolites eventually produces a distortion of the cell followed by rupture, either *in vitro* or immediately after transfusion. Cooling and freezing also are known to be detrimental to the lipo-proteins of plasma, some of which seem to interact with the erythrocyte membrane. Cooling may in this fashion initiate a nonreversible pattern of deterioration.

Since most of the metabolic enzymes require specific cations for efficient operation, another approach to the control of cell metabolism during storage might be the removal of the ions necessary for enzymic action. Work with ion exchange agents[14] has resulted in great improvement in the maintenance of the integrity of certain plasma proteins, leukocytes, and platelets, but as yet has yielded little or no improvement in erythrocyte aging. It is probable that one cannot influence the interior of the cell by this means sufficiently to be of great value. A more successful attempt at this approach may be forthcoming from the work with chelating agents some of which may actually penetrate the erythrocyte membrane. One limitation of this approach may be the dependence of the cell membrane on some of the same cations removed. Cell physiology has shown the dependence of cellular membranes generally on the presence of calcium.

It might be presumed that the colloid osmotic pressure of the plasma proteins would offer important protection in the prevention of the distortion of the cell produced during storage. It can be shown in studies with resuspended cells, however, that certain plasma proteins, present in amounts affording colloid osmotic pressure equivalent to that of plasma, offer little protection beyond that provided by isotonic solutions of crystalloid. Other proteins, such as modified globin or a plasma lipo-protein, offer protection completely without relation to their colloid osmotic effect, suggesting an interaction with the cell membrane as the factor determining the value of proteins in cell storage.

The very first problem to be met in all blood storage—keeping the blood in a fluid state—also may influence the subsequent durability of the blood cells. Although careful attention to the use of nonwettable surfaces will permit the collection of blood that does not clot rapidly, for the purposes of storage one must still resort to some form of anticoagulant. Usually this has meant the use of an agent that will bind cationic calcium which is essential in coagulation. At the moment, citrate ion remains the agent of choice for this purpose, citrate being less toxic than oxalate and less expensive than heparin. Citrate, however, interacts with many plasma proteins, has an extremely toxic effect on white cells,[15] and produces some disturbance of the metabolism of erythrocytes. Another type of chemical binding agent, ethylene-diamine tetraacetic acid (Versene, Sequestrene, EDTA), has been employed by Proescher as an anticoagulant[16] and is being extensively studied at present with promise of improvement over citrate. Ideally, one might avoid the use of any adulterating agent, if possible. An approach to this has been made in the use of ion-exchange resins, as mentioned above, which remove the cations rather than binding them.

This brief statement of the problems to be handled in the storage of blood will furnish a background against which we may consider the accomplishments of the past few years in this field.

Smith, Sloviter, and associates,[17] following the observation that sperm can be preserved for extended periods by freezing in the presence of glycerine, have applied a similar technic to whole blood. Blood frozen rapidly at —79 C. in the presence of an equal quantity of 30 per cent glycerine has been held at this temperature for 42 days, then thawed and transfused with normal survival of those transfused cells which are not lost during the freezing and thawing. This demonstrates that by proper cooling one can suffi-

ciently slow metabolic rates to produce a real extension of storage life. The present limitations of this technic are due to the loss of cells during thawing, which may be extensive in the longer storage periods, and to the difficulty of removing the glycerine before transfusion. This is presently being done by dialysis against graded concentrations of glycerine. Corroboration of this work has not yet appeared in the literature.

FIG. 1

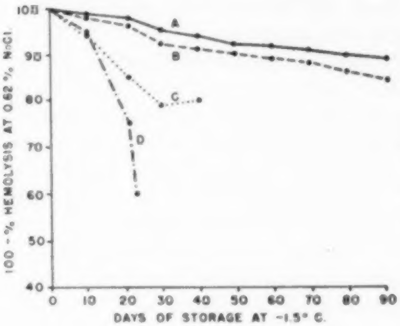

FIG. 2

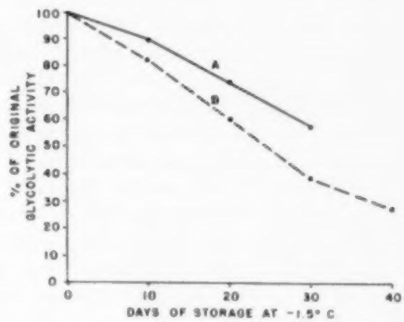

Fig. 1. Osmotic fragilities of cells stored as:

A. Cells suspended in 4% modified globin, 5% lactose, 0.25% dextrose collected by method of Strumia et al. (Average of 6 studies. Spread of values at 90 days 80-92%.)

B. Whole blood collected by method of Strumia et al. (Average of 8 studies. Spread of values at 90 days 81-93%.)

C. Cells suspended in 4% modified globin, 5% lactose, 0.25% dextrose collected in ACD solution (N. I. H. formula B) without refrigeration during collection.

D. Whole blood collected in ACD solution (N. I. H. formula B) without refrigeration during collection.

Fig. 2. Glycolytic activity of cells stored as:

A. Whole blood collected in ACD solution (N. I. H. formula B) without refrigeration during collection.

B. Cells suspended in 4% modified globin, 5% lactose, 0.25% dextrose collected by method of Strumia et al. (Average of 4 studies. Spread of values at 40 days 19-48%.)

Strumia reported an extension of the life of whole blood and a still greater extension of the life of resuspended red cells.[18] This was accomplished by collection of the blood in isotonic neutral citrate solution, with careful admixing and refrigeration during the collection, and storage at 1C. Within 12 hours of collection, lactose, dextrose, and citric acid were added to the whole blood, or lactose, dextrose and modified globin were added to red cells in a quantity equivalent to that of the plasma withdrawn. Studies employing the technics suggested by Strumia have been performed in our laboratory indicating a real improvement in the structural integrity of cells so handled (figure 1). Attempts to repeat the resuspension technic of Strumia in at least two other laboratories have failed to indicate satisfactory survival of the transfused cells after storage for longer than 21 days.[19, 20] Careful examination of our data in the light of these reports indicates that the glycolytic activity of the stored cells at 29 days calculated as per cent of the original

glycolytic activity at the time of collection gives a figure corresponding reasonably to the per cent survival found after transfusion. These data check both with the observations of failure to find a general lengthened storage life of the resuspended cells and with the erratic behavior of individual transfusions later noticed by Strumia himself (figure 2). It is noteworthy that, at 21 days' storage, the data from both osmotic fragility and glycolytic measurements suggest about 70 per cent survival of the erythrocytes from blood collected in ACD solution. This percentage accords well with published data for *in vivo* survival of such cells transfused after 21 days storage.

Proescher[16] has reported successful substitution of the chelating agent, ethylene diamine tetraacetic acid, for ACD solution as an anticoagulant. Substitution of 0.3 per cent or 0.6 per cent solutions of EDTA in isotonic saline for neutral isotonic citrate solution as an anticoagulant, but otherwise following the technic of Strumia, has yielded laboratory data suggesting improvement in the aging of whole blood as compared to blood collected in ACD solution (figure 3). These data suggest the possibility of a useful storage life up to 40 days for blood collected with EDTA as the anticoagulant.

Search for a plasma protein which might stabilize erythrocytes by specific interaction with the erythrocyte membrane has been extensive. Gibson found evidence of such a protein in Fraction IV-3, 4,[21] but his work has never been extended. Recently, in Fraction III-1, such a protein seems to have been found in our laboratory. Identity of the protein has not yet been established, but it appears to be a lipo-protein which could readily be made available during routine plasma fractionation (figure 4).

One major difference between blood in the bottle and blood in the body is the repeated exchange of carbon dioxide for oxygen in the lung. Attempts to oxygenate blood during storage have suggested that this may be a very hopeful technic and may provide the key to correlation of data from studies which have hitherto been baffling (figure 5). Present effort is being devoted to devising a means of ready continuous oxygenation during storage so that this phenomenon may be accurately evaluated.

In summary, although we cannot begin tomorrow to keep blood for months, technics may have already been reported that will be shown by corroborative studies to provide for a major extension of our ability to keep both whole blood and resuspended cells.

Fig. 3

A. Osmotic fragility of cells stored as whole blood collected in 100 ml. 0.6% EDTA, 0.65% NaCl. Ordinate 100-% hemolysis at 0.62% NaCl.

B. Osmotic fragility of cells stored as whole blood collected in 100 ml. 0.3% EDTA, 0.65% NaCl. Ordinate 100-% hemolysis at 0.62% NaCl.

C. Glycolytic activity of cells stored as whole blood collected in 100 ml. 0.3% EDTA, 0.65% NaCl. Ordinate % of original glycolytic activity.

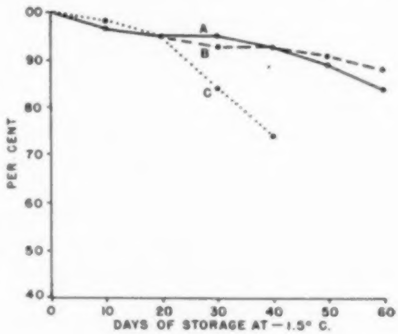

Fig. 3

There is new hope for a reasonably accurate correlation of laboratory and clinical observation in this field. Such a correlation should immeasurably facilitate and speed research.

Fig. 4

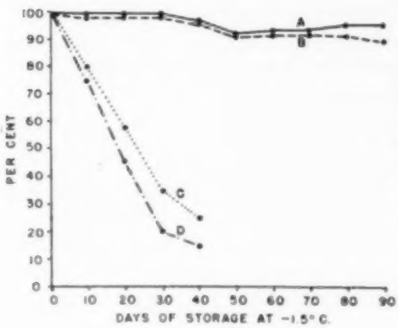

Fig. 5

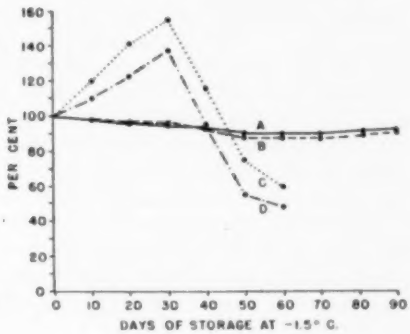

FIG. 4

A. Osmotic fragility of cells resuspended in 2% Frac. III-1, 5% lactose, 0.25% dextrose. Ordinate 100-% hemolysis at 0.62% NaCl.

B. Osmotic fragility of cells from same bleeding as A resuspended in 4% modified globin, 5% lactose, 0.25% dextrose. Ordinate 100-% hemolysis at 0.62% NaCl.

C. Glycolytic activity of cells resuspended in 2% Frac. III-1, 5% lactose, 0.25% dextrose. Ordinate % of original glycolytic activity.

D. Glycolytic activity of cells from same bleeding as C resuspended in 4% modified globin, 5% lactose, 0.25% dextrose.

FIG. 5

A. Osmotic fragility of cells stored as whole blood under oxygen, collected by method of Strumia et al. Ordinate 100-% hemolysis at 0.62% NaCl.

B. Osmotic fragility of cells from same bleeding as A stored as whole blood under air. Ordinate 100-% hemolysis at 0.62% NaCl.

C. Glycolytic activity of cells resuspended in 4% modified globin, 5% lactose, 0.25% dextrose, stored under oxygen. Ordinate % of original glycolytic activity.

D. Glycolytic activity of cells from same bleeding as C, resuspended in 4% globin, 5% lactose, 0.25% NaCl, stored under air. Ordinate % of original glycolytic activity.

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Recent Advances in the Preparation of Stable Plasma Derivatives

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"In any blood program, the protection of the donor and of the recipient is the obligation of the clinician. The opportunity of the chemist begins as the blood enters the needle inserted in the vein of the donor and continues until the final product enters the needle to be transfused into the recipient; without virus, pyrogens, antigens, depressor or any untoward reaction. During the interim, the choice of equipment, of anticoagulant, of method of processing, of temperature and conditions of storage raises many problems in biologics rather than in medicine."[1]

The preparation of purified proteins from human plasma, begun in 1940 and successfully developed into large scale during the war, led to many advances, not only of theoretical interest but also of practical value.

To the chemist these researches proved the feasibility of separating plasma into its component parts and demonstrated that no obstacle existed to the separation and stabilization of any protein from any tissue. Plasma proteins, available in good yield in highly purified form, could be rendered far more stable than in whole plasma. Moreover, there emerged from these studies important contributions to our knowledge of the state of the proteins, not only of plasma but also of the tissues, of the interactions of proteins, and of their structure.

To the cytologist, these purified plasma proteins opened up new avenues for the investigation of interactions with cells, interactions which have led not only to improved preservation of blood cells but also to an understanding of the nature of physiologic interactions of the blood cells.

To the immunologist, they provided, for the first time, concentrated human antibodies, which led to advances in the knowledge of the nature of antibodies and of their interactions with antigens.

To the epidemiologist "the concentrated antibodies, separated from the pooled plasma of a population, offer a means of characterizing the state of immunity of that population as a permanent public health record. Were such records systematically collected and available, it might prove possible to follow the course of epidemics much as the course of a comet may be followed by the photographic records of the skies, recorded by modern observatories. Not infrequently the first faint trail of a comet has escaped

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detection, and the origin and course have been understood only because astronomers now map all of the heavens systematically at all times. The systematic collection and preservation as dry white powders of the concentrated antibodies of diverse populations might comparably simplify the analysis of the course of certain epidemics."[2]

To the physician, these stable plasma derivatives, available in compact form, have proved invaluable in the management of many pathologic situations. Indeed, in many cases, the purified proteins have proved more effective than whole blood or plasma. The availability of the immune globulins, in concentrated form, and of reproducible quality and potency of the measles antibodies, has meant that the clinician can adjust the dose and, with a high degree of accuracy, modify the course of measles rather than completely prevent the disease.

THE VIRUS PROBLEM

One of the most serious problems arising out of large-scale transfusions is the increased danger of transmitting disease from a donor population to a susceptible recipient population. This is particularly true of diseases of virus origin; the increased incidence of serum hepatitis is one result. The risk of transmission of the hepatitis virus accompanies the use of all blood derivatives. In the case of the albumins and immune globulins, however, this risk has never materialized; these useful plasma proteins have, from the beginning, been free of contamination by the serum hepatitis virus.

This long record of safety, recently reviewed critically by Drs. R. S. Paine and C. A. Janeway[3] in a study of a large number of infusions of human serum albumin, distinguishes these blood derivatives from most others now available. Two factors may have contributed to this. Although there is no evidence that the hepatitis virus is present in the albumin fraction separated from pooled plasma, the albumins proved so stable that they could be heated for 10 hours at 60 degrees in the final container, one of the few treatments known to inactivate the hepatitis virus.[4] The second and more likely reason for the excellent record of these blood derivatives lies in the properties of viruses themselves. Viruses are proteins and are probably concentrated, as are the other proteins, into a single fraction. On the basis of clinical evidence, it always has been assumed that the hepatitis virus was concentrated into the fraction containing fibrinogen and the antihemophilic globulin; recently it has been suggested, from clinical evidence, that this fraction now in great demand for the treatment of fibrinogen-deficient states can also be rendered free of the danger of virus transmission.[5]

BLOOD ECONOMY

The supply of blood is not inexhaustible; in order to meet the continually increasing demand, the greatest economy must be exercised in using it; every useful property must be taken advantage of. It is a dissipation of valuable assets to use whole plasma to restore colloid osmotic pressure; it is a waste of valuable therapeutic properties to use whole plasma to overcome a deficiency of a single component, such as occurs, for example, in hemophilia. It is an extravagance to dry plasma and damage the labile lipoproteins, or to subject plasma to the destructive effects of various viricidal agents which bring about

deep-seated changes to many valuable proteins. Indeed, the need for even greater blood economy is emphasized by the present situation, with respect to the immune globulin fraction. Should this fraction, the supply of which barely exceeds the demand for use in measles, be found useful in the control of other infectious diseases, a drastic upward revision in the production of these components of plasma would be necessary. It is even conceivable that only by subfractionating this concentrate in the interest of obtaining specific antibodies could such a demand be met.

The greatly accelerated efforts during the war produced many important advances in blood technology. We saw the stock-piling, for the first time, of a few blood derivatives; we saw the purification of a few important plasma proteins and realized their great stability following purification. We saw, before the end of the war, the preservation of whole blood long enough to render it useful in military operations. And we saw, as a result of these advances, the magnitude of the salvage program that is an inevitable consequence of these developments. However important these advances were in terms of military medicine or of public health, their greatest significance lay in the lessons they taught for the future. To have been satisfied that they could form the basis of a lasting blood program was just as erroneous as to expect scientists ever to be satisfied when the first few promising results are obtained in research.

BIOCHEMICAL AND BIOMECHANICAL PROCEDURES

Even during the war, a keen awareness of the inadequacies, not only of the processing procedures which had been developed and used so successfully but also of the methods of blood collection themselves, characterized the feelings of the group at Harvard, and especially of Dr. Cohn. Many changes in the labile proteins of the plasma had taken place as a result of the long time which intervened between the collection of the blood and the start of processing. The methods and reagents employed in the process were early recognized as responsible for further degradative effects, but under the conditions of urgency which were obtained, it was not possible to make any but minor modifications and improvements so as not to interfere, even for a short time, with the production of serum albumin.

In the intervening years, we have passed rapidly through a series of important developments that have culminated in a new chemistry of biologic products and a new system of biomechanical apparatus, and which have already begun to replace the older inadequate methods. One of the key developments was the introduction of the exchange resin for blood collection by Drs. Walter, Gibson, and Buckley.[6] Not only did this make possible the collection for the first time of white cells and platelets in a viable state, but the plasma from blood collected in this way proved far superior to citrated plasma, and more nearly in its state of nature than was possible of attainment by any of the earlier methods. We quickly recognized that we had never before seen certain of the labile proteins, including some of the important lipoprotein constituents of plasma, in anything but a denatured state.

At Dr. Cohn's insistence, the new developments have been pursued side by side. Although the engineering aspects of the development of mechanized equipment for

blood collection and processing in a closed sterile system have given rise to a general impression that this was the principle achievement, they are, in fact, wholly dependent on, and supplementary to, the far-reaching fundamental developments in the chemistry of natural products which have been, and continue to be, the objects of the greatest effort. Indeed, the biomechanical equipment would not have been possible without the chemical knowledge gained from the work of the large group of investigators at the University Laboratory and of their collaborators not only in Boston but elsewhere.

Specific chemical interactions form the basis of natural processes. It is the spatial arrangement of the functional side chains of the amino acids, projecting peripherally from the backbone of the protein molecule, which imparts to these important natural products their specific chemical and physiologic properties. It is because of such a steric configuration that the metal combining protein, alone of all the proteins of plasma, reacts with iron and transports this important metal in tight combination. It is through such specific sites that the metal activated enzymes interact with their substrates. The complex reactions leading to blood coagulation and the formation of antigen-antibody complexes are but a few examples of the highly specific protein-protein interactions which are continually encountered in natural systems.

There is, in addition, a property common to every protein which we have studied, not only from plasma, but from tissues and embryonic fluids as well. The orientation of the functional groups in proteins, as well as the chemical nature of these groups, of the amino groups, of the sulfur-containing groups, of the acid groups, and of the other types of groups, results in a spectrum of reactivities with a wide variety of metallic ions. These metals include, for example, calcium, magnesium, manganese, copper, cobalt, iron, and zinc, all of which occur in nature. At one end of the spectrum are the proteins to which the metals are so firmly attached that rupture of the molecule is necessary to effect their release; this group includes the heme proteins and the recently isolated copper protein, caeruloplasmin.[7] Then there are the metal activated enzymes, already referred to, in which the association constant of the reaction between the protein and the metal may range from extremely high to relatively low values; many of these enzymes may be reversibly inactivated by dissociating the metal from the protein. At the other end of the spectrum are the alkaline earth metals, calcium and magnesium. Here the association with the proteins is weak; rapid passage of blood over the exchange resin in blood collection results in essentially quantitative removal of these metals.

INTERACTIONS WITH ZINC

Zinc ions form complexes with proteins by interaction with the imidazole groups of histidine residues.[8] In most cases the resulting complexes are less soluble than the corresponding sodium salts. When zinc is added to human plasma, roughly one third of the proteins precipitate. Moreover, the separation between insoluble and soluble zinc complexes is quantitative with respect to most of the known components of plasma, as shown in table I. Those proteins forming soluble zinc complexes include the serum albumins, the metal combining protein, the α-lipoprotein, and certain α_2-globulins. The precipitate is rich in globulins and contains most of the labile components of plasma, such as those

TABLE I

Separation of the Protein Components of Human Plasma

	Components Separated		Amount per liter of plasma
PLASMA from blood collect over cation exchange resin	Prothrombin, S.P.C.A. precursor* leucocyte-phagocytosis stimulating factor, unstable lipoproteins		0.4 g.
Solid $BaSO_4$	Isoagglutinins		(0.1 g.)
Erythrocyte Stroma PLASMA GLOBULIN PRECIPITATE From aqueous solution with 15 mM zinc per liter of plasma at pH 7.4, 0°C.	Fibrinogen Cold insoluble globulin Antihemophilic globulin Accelerator globulin Plasmin inhibitor	Plasminogen Immune globulins β-Lipoproteins β_2-Globulins Caeruloplasmin	24 g.
Cation Exchange Resin to remove zinc STABLE PLASMA PROTEIN SOLUTION 5.5 per cent proteins in Native Sodium Salts of Blood	Serum albumins α_1-Lipoproteins α_1-Bilirubin globulin α_2-Glycoprotein β_1-Metal-combining globulin	Acid glycoprotein Alkaline phosphatase Plasma esterase Amylase Iodoprotein Mucoprotein	900 ml.

* S. P. C. A. precursor refers to the precursor of serum prothrombin conversion accelerator.

involved in blood coagulation, as well as the immune globulins, the β-lipoproteins, and many other important proteins present in small amounts.

S.P.P.S.

The solution remaining after removal of the Plasma Globulin Precipitate, freed of zinc by passage over a carboxylic type exchange resin, is essentially plasma from which a third of the proteins has been removed. Because the exchange resin used in the collection of the blood avoids the dilution of the plasma encountered with the usual anticoagulants, the protein concentration of the solution is about 5.5 per cent. Because the proteins which are left in solution, 70 per cent of which are serum albumins, are among the smaller proteins of plasma, the solution retains approximately 80 per cent of the colloid osmotic efficiency of plasma.

If the plasma were treated with barium sulfate, as described below, before separation of the insoluble zinc-globulin complexes, this solution proved to be extremely heat stable, a property which suggested its name, Stable Plasma Protein Solution (S.P.P.S.). In the presence of a small amount of sodium caprylate it can be heated for 10 hours at 60 degrees, without detectable change to the proteins, with the exception of a decreased extractability of the cholesterol from the α-lipoprotein. S.P.P.S. is now undergoing limited clinical trial.

This single simple chemical operation thus makes possible the separation from plasma of the components, such as the gamma globulins, antihemophilic globulin, fibrinogen, pro-

thrombin, accelerater globulins, plasminogen, and caeruloplasmin, which have valuable properties in the treatment of pathologic conditions and which must be preserved in the interests of blood economy. At the same time it yields a solution, with only slightly reduced colloid osmotic efficiency, which can be rendered safe from the danger of virus transmission and can be stockpiled in the liquid state without refrigeration.

The simplicity of this separation, and of the others which are subsequently carried out, is startling, especially to those familiar with the complexities of the older methods. The whole operation is carried out at physiologic pH, and without dilution of the plasma. The zinc replaces the alcohol and low temperatures of the earlier processes, and the costly methods of drying from the frozen state are completely eliminated. Moreover, these operations can be completely mechanized in automatic equipment.

Separation of purified proteins from the Plasma Globulin Precipitate is achieved by fractional extraction. Differential solubility of the various proteins is accomplished by selectively dissociating the zinc complexes, one by one, using reagents which compete with the protein for the metal. The affinity of these reagents for the metal must be near that of the protein, otherwise complete dissociation occurs and all the proteins pass into solution. The amino acids have been used in these systems, since reagents, such as citrate, with higher affinity for zinc are too strong and completely redissolve the zinc protein complexes.

INTERACTIONS WITH MERCURY

So far we have discussed the metals which are encountered in the specific chemical interactions of natural processes. Let us turn now to the metals such as mercury and barium which have heretofore been regarded as poisons. These metals generally exert their harmful influence by displacing the natural metals from specific proteins, or by blocking groups, such as the sulfhydryl groups, which are essential in biochemical reactions. If used without interfering with specific mechanisms, and if removed quantitatively at the end of processing, the chemist can take advantage of a large number of specific interactions of the heavy metals.

Various situations, such as the necessity of salvaging albumin and other proteins from the plasma separated from outdated blood, will always demand further purification of the proteins which form water-soluble zinc complexes. The solution to this problem, without having recourse to the use of alcohol, was found by Dr. Karl Schmid,[9] who showed that zinc-mercury complexes of these proteins are completely insoluble in water. The solution remaining, after removal of the precipitate, fails to yield any additional protein when tested with trichloracetic acid. Indeed, a reagent containing both zinc and mercury precipitates all proteins from plasma without denaturation. Fractional extraction of the zinc mercury complexes can be carried out by taking advantage of the same principles which were found useful in obtaining purified proteins from the Plasma Globulin Precipitate. The study of this system, carried out largely by Dr. William H. Batchelor,[10] has already yielded extremely interesting preparations of purified α-lipoprotein as well as of serum albumins.

INTERACTIONS WITH BARIUM

Specific separations also have been obtained using other metals, including barium, cadmium, nickel, and lead. Barium, for example, has been used both in solution and in the

solid phase. Treatment of plasma with barium sulfate, or with a cation exchange resin whose reactive sites have been saturated with barium ions, results in the removal of a small fraction, comprising less than 0.5 per cent of the plasma proteins, which contains all the prothrombin and precursor of serum prothrombin conversion accelerator, as well as the extremely interesting protein, recently discovered, which stimulates phagocytosis by isolated leucocytes.[11,12] These proteins may be subsequently recovered by treatment of the barium sulfate complex with sodium citrate, which displaces the proteins from their state of association with barium.

ANTIGEN-ANTIBODY INTERACTIONS

One further interaction should be mentioned. An insoluble antigen, the stroma of the red cells, has been used to separate and purify an antibody, in this case the isoagglutinins, from plasma. The advance which made possible utilization of this well-known interaction came from the theoretical investigations of Dr. Henry Isliker,[13] who not only found conditions for preparation of the stroma in such a way as to render them applicable to continuous use in simple columns but also found conditions for eluting the isoagglutinins from the stroma, using the carbohydrates, lactose, galactose or glucosamine. The implications of this technic, not only in the preparation of highly purified isoagglutinins and in the development of rapid technics for blood typing but also as a general method for concentrating soluble antibodies, are obvious and are now being extended.

INTERACTIONS WITH VIRUSES AND TISSUE PROTEINS

Because they make use of reactions similar to those found in nature, these specific biochemical tools for the separation of proteins are equally applicable to the study of other tissues and body fluids. They have already proved extremely useful, for example, in the studies of the proteins of liver.

We have already referred to the practical problems arising from the danger of transmitting the hepatitis virus in blood derivatives. Because viruses are proteins, the destruction of viruses with various chemical and physical agents has generally been accompanied by extensive damage to other proteins as well. On the other hand, even in the earlier methods the virus was concentrated during the fractionation. This attack on viruses, namely their concentration from tissues rather than their destruction, is now being pursued by Dr. Cohn and his collaborators. At least three different methods for concentrating viruses by specific chemical interactions now appear promising. In addition to the opportunities thus afforded for the study of viruses, these methods may also prove applicable in rendering cellular components free of viruses. It has already been shown[14] that certain viruses can be concentrated from infected tissues as metal complexes and that, following dissociation of the metal-virus complex, the virus is obtained without loss of yield or potency. This in itself is a tribute to the versatility of these interactions; furthermore, it constitutes additional proof, if any skepticism still remains, of the mildness and safety of these specific chemical interactions.

The importance of ion exchange resins in effecting the quantitative reversal of metal-protein interactions cannot be sufficiently stressed. Without the versatile exchange resins, these interactions would probably remain laboratory curiosities.

These are but a few examples of the usefulness of the new biochemical methods in obtaining purified plasma proteins. While they greatly simplify the preparation of the proteins which have been available since the war, and with which most clinicians are familiar, their greatest value undoubtedly lies in the preparation of the many physiologically important proteins which have not heretofore been possible to obtain in a natural state. Already it appears that in addition to S.P.P.S. and the immune globulins, the components of the clotting mechanism, including prothrombin and the accelerator globulins and many other proteins of equal importance, can be made available, in the interests of blood economy, in stable states, free from the danger of virus transmission. Moreover, there is no longer any doubt that the methods are now at hand to resolve the gamma globulin antibodies, a problem of considerable interest to the chemist and of great practical importance if the supplies of these important proteins are to meet the demands imposed by expanding experience.

These chemical methods and the biomechanical equipment which they make possible have, however, even greater potentialities. It is now possible to obtain separated erythrocytes, leucocytes, platelets, and stable plasma derivatives, using portable equipment in the field, within a matter of minutes after the blood has left the vein of the donor. It is now possible to enter an infectious ward and collect and process blood, without danger to personnel, because the operations are carried out under sterile conditions, in the interest of studying the interactions of viruses and other infectious agents. It is now possible to prepare hyperimmune globulins for the control of infections against which the normal immune globulins are ineffective, by collecting blood in the field from donors convalescing from disease.

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Clinical Status of Plasma Fractions

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It is a pleasure to participate in this symposium. Frequently during the past ten years, the group of investigators to which I have belonged has collaborated with members of the research staff of Sharp and Dohme, as have other groups interested in the blood transfusion problem. I feel sure that the opening of these new laboratories will potentiate the contributions which research workers here, on their own, and in collaboration with their colleagues in other laboratories, can make to blood technology and thus to the public health.

Dr. Surgenor has discussed the rationale of plasma fractionation from the standpoint of the chemist concerned with the separation, stabilization, and preservation of each protein in as close to its natural state as possible. I shall discuss these separated proteins from the point of view of the physician who wishes to apply them to the treatment of his patients, of the public health worker who is interested in the prevention of disease, and of the clinical investigator who desires to understand the molecular basis of normal bodily functions and their disturbances in disease. For all these purposes, certain requirements must be met. The protein preparations must be of known and reproducible potency; they must be relatively pure and hence specific; they must be available when needed; and, above all, they must be safe. These criteria have been met to a considerable extent in the case of products prepared by the standard alcohol-water method of fractionation; it is our expectation that they will be met even more fully with products prepared by the newer methods, based on interactions with metals. Unfortunately, the technics of clinical investigation are less exact than those of chemistry, and it will probably be many years before the full story of the clinical value and physiologic role of any protein fraction can be told. However, during the past decade an impressive body of knowledge about these products has accumulated, and there can be no doubt that they are important therapeutic agents, effective weapons for the control of certain common infectious diseases, and potent tools for clinical and physiologic investigation.

SERUM ALBUMIN

The development of serum albumin was predicated on the well-documented assumption that restoration of the volume of circulating plasma was the most urgent need in cases of traumatic shock and that the safest and most effective agent for that purpose would be the natural protein responsible for most of the colloid osmotic pressure of plasma.[1] A

Given at a Symposium on "Frontiers of Research on Blood and Plasma Extenders," marking the formal dedication of the new Sharp & Dohme Medical Research Laboratories, West Point, Pennsylvania, May 12, 1952.

great deal of misunderstanding has arisen in the past because of failure to appreciate that packaging of albumin as a 25 per cent solution was for logistic and not for physiologic reasons.[2] The patient in shock from loss of blood or plasma loses not only colloids but the electrolytes and water in which those colloids are dissolved. Obviously, replacement therapy will be more efficacious if electrolytes and water as well as protein are administered. Serum albumin is effective in restoring lost blood volume in cases of experimental and clinical shock and behaves predictably in terms of its known osmotic and other physiochemical properties (figure 1).[3] In view of the safety, convenience, and value of serum albumin as an emergency replacement fluid, in instances of severe injury or blood loss, I hope the day will come when every practicing physician will be able to carry one or two packages in his bag. The fact that the patient receiving serum albumin is safe from the risk of homologous serum hepatitis recommends it particularly as the emergency transfusion fluid of choice.[4]

Serum albumin, with a net charge higher than that of most of the other plasma proteins, is known to interact with, and bind many, small molecules and ions. Thus, a number of drugs, such as the antimalarials and sulfonamides, fatty acids, and other substances such

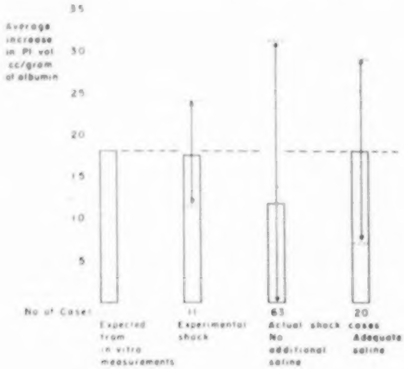


FIG. 1. The average effect of normal serum albumin on plasma volume in cases of experimental and clinical shock. The height of the bar indicates the average increase in plasma volume (in cubic centimeters) per Gm. of injected albumin in each group of cases. Arrows with dotted lines at end indicate range of values in each group. From left to right, first bar shows expected plasma volume increase (18 cc./Gm.) from *in vitro* measurements of colloid osmotic pressure of albumin, second bar indicates results in a group of volunteers in whom albumin infusion followed measured blood loss by venesection, third bar indicates results in a large group of cases of traumatic and hemorrhagic shock to whom little or no saline was given in addition to concentrated albumin, and fourth bar gives results in a group of similar shock cases to whom an ample quantity of saline was given in addition to the concentrated albumin infusion. These data were collected for the Committee on Medical Research of the Office of Scientific Research and Development by four groups of investigators: Dr. E. A. Stead and his colleagues at Emory University, Atlanta; Dr. J. Rhoads and his associates at the University of Pennsylvania, Philadelphia; Dr. Everett I. Evans and his group at the Medical College of Virginia, Richmond, and Drs. D. W. Richards and Andre Cournand at College of Physicians and Surgeons, Columbia University, New York.

as thiocyanate ion, are bound in varying degrees to albumin—a fact which determines their transport in the blood and distribution in the body fluids.[5] Our knowledge of the clinical significance of such interactions is rudimentary. The role of serum albumin in the total protein economy of the body, its intermediary metabolism, and its relation to the formation of such tissues as bone matrix are incompletely understood.[6] It seems very likely, however, that as our knowledge is extended, differences between the natural proteins and the foreign colloids under consideration as "plasma expanders" will become increasingly evident.

A-282736 Eczema with Hypoproteinemia

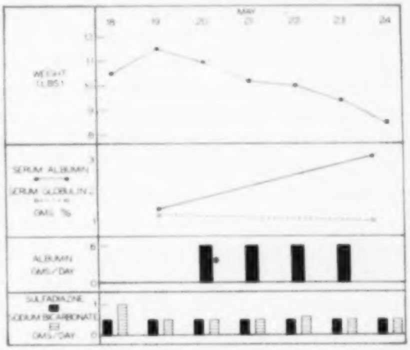


FIG. 2. Delivery of edema (as shown by fall in body weight) from an infant with infected weeping eczema and severe hypoalbuminemia during administration of 24 Gm. of albumin in four days. At the end of this time, infant was free of edema, infection was under control, and infant began to take food eagerly. (Reproduced from J. A. M. A. *126*:676, 1944, with permission of author and publisher.)

Specific deficiency of serum albumin occurs frequently in medical and surgical patients. A number of factors may produce it: inadequate intake or assimilation of food, loss of albumin in exudates, transudates, or the urine as in the nephrotic syndrome, increased catabolism, or inadequate synthesis to equal the rate of degradation at normal concentrations of serum albumin in the blood. At any given level of circulating albumin, a complex series of equilibria maintain blood volume and govern the distribution of fluids and electrolytes in the body and their excretion by the kidney. If the level of albumin is low, this homeostatic balance may only be achieved at a price—massive edema. The extent of the participation of the central nervous system, the kidney, and the endocrine system, particularly posterior pituitary and adrenals, in regulation of these equilibria is only beginning to be appreciated.[7] It is not surprising that there is no general agreement on the exact place of albumin in the treatment of hypoproteinemic states. It certainly should be administered when hypoalbuminemia must be quickly repaired, as in preparation of an edematous, hypoproteinemic patient for surgery or in support of such patients during episodes of stress, infection, or in the postoperative period (figure 2). Patients with gastrointestinal cancer, ulcerative colitis, severe burns or other weeping lesions of skin, or hepatic or adrenal insufficiency frequently require such supportive treatment. Experience has shown that, even though serum albumin may be administered in concentrated solution with little additional fluid or salt, there is a definite limit to the rate at which the readjustments accompanying a rise in serum albumin concentration can be made. This depends on the ability of the circulation to cope with the increase in plasma volume following each injection, an increase which is gradually dissipated through excretion of fluid by the kidneys and by shunting of fluid into the extravascular compartment. In order to raise the

serum albumin concentration appreciably, several times as much albumin must be infused as would be predicted from calculations based on plasma volume.[8] Thus the "albumin space" is much bigger than the measured plasma volume.

Two chronic diseases, cirrhosis of the liver and the nephrotic syndrome, are regularly associated with a decrease both in the concentration and total amount of circulating albumin. Despite a number of studies on the effects of daily administration for a considerable period in order to relieve the ascites and edema so characteristic of these conditions, observers are not unanimous as to its value or deleterious effects. In early cases of cirrhosis, albumin injections seem to accelerate the onset of the diuresis, which usually occurs during a period of hospital treatment.[9] In advanced cases, there is some risk of rupture of esophageal varices, and the result of albumin administration may be only a parallel rise in colloid osmotic pressure of both blood and ascitic fluid without diuresis.[10] Although daily intravenous albumin injections are well-tolerated by most patients with the nephrotic syndrome, and a diuresis occurs in about half of patients so treated,[11] the discomfort of the daily injections, the loss of most of the injected protein in the urine, and the possibility of renal damage from the massive proteinuria had already tended to discourage its use prior to the introduction of corticotropin or cortisone.

Local edema has offered a tempting therapeutic target for concentrated albumin, despite the obvious fact that, if edema is localized, a local increase in vascular permeability is its likely cause. Results of albumin administration in instances of cerebral edema, localized edema of the gastro-intestinal tract, and inflammatory pulmonary edema have not been uniform, although occasionally clinical results are dramatic.

As a tool in the hands of the clinical investigator, serum albumin has been of great value. As a safe, well-standardized agent for producing a predictable increase in plasma volume it has been used a great deal in studies of the regulation of the circulation. Basic investigations of the hemodynamics of shock and of the role of the brain and kidney in the adjustment of body fluid volume have been made possible. The administration of albumin to patients with cirrhosis of the liver has helped to clarify concepts of the mechanism of ascites. The fact that patients with the nephrotic syndrom lose injected normal albumin almost quantitatively in the urine has strengthened the concept that the disease primarily involves the kidney rather than the plasma proteins. Many interesting metabolic studies which have contributed to our knowledge of protein and calcium metabolism could hardly have been performed without such a safe solution available as a source of parenterally administered protein. Recently serum albumin, labeled with radioactive iodine, has been used for plasma volume measurements and as a tool for study of the rate of albumin turnover in normal subjects and patients with cirrhosis of the liver.[12]

S.P.P.S.

Stable plasma protein solution (S.P.P.S.), already described by Dr. Surgenor as the solution remaining after the globulins have been precipitated from plasma with zinc in the new fractionation process, is a 5.5 per cent solution of albumins and more soluble globulins in the plasma electrolytes. Theoretically, it should be more satisfactory than albumin for all uses where volume replacement rather than specific repair of hypoalbuminemia is desired, except in those situations where the compactness of the albumin

package possesses logistic advantages. Clinical trials have been too meager as yet to allow conclusions, although they seem promising. The fact that S.P.P.S., like albumin, can be heated for 10 hours at 60° C. makes it possible to use it with considerable assurance that it will not produce homologous serum hepatitis.

BETA-METAL-COMBINING GLOBULIN

Fraction IV-7, which contains this metal-combining globulin in high concentration, has not been used as a therapeutic agent because no instances of a specific deficiency of this protein have yet been found. Serving as the normal vehicle for transport of iron in the plasma, it is of great physiologic interest and has been employed by a few investigators in studies of iron metabolism.[13]

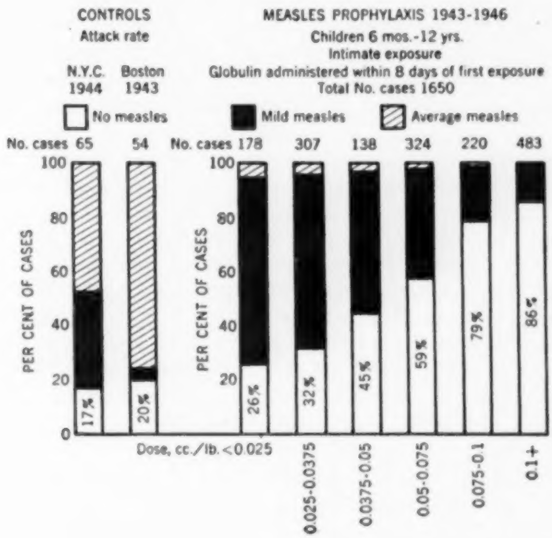


FIG. 3. Summary of results with gamma globulin in the prophylaxis of measles. Note correspondence between size of dose and results in intimately exposed children between 6 months and 12 years of age. This analysis has led to recommended dosage: 0.1 cc./lb. for prevention; 0.02 cc./lb. for modification. (Reproduced from Advances in Internal Medicine, Vol, III, p. 328 with permission of author and publishers.)

SERUM GAMMA GLOBULIN

The gamma globulin fraction, in which almost all the antibodies from large pools of normal human plasma are concentrated 20-25 times, has been used the most extensively of all fractions.[14] Since the demonstration in 1943 that it was a safe, effective agent for the prevention or modification of measles, an estimated over two million doses have been given in this country.[15] Administration of gamma globulin in small doses to exposed

susceptible children so that they may develop a mild attack and thus acquire a lasting immunity without serious risk has become standard practice (figure 3).[16]

With growing appreciation of the potential seriousness of infectious hepatitis, the proof that gamma globulin would prevent or modify this infection in exposed individuals has opened up another large demand for this valuable agent (figure 4).[17]

Measles and epidemic hepatitis are the only two viral infections of man in which it has been proved that the level of antibody is sufficiently high in the blood of the average normal adult to yield an adequately potent gamma globulin preparation. In the case of chicken pox, mumps, and german measles, diseases which also give a lasting immunity, this is not the case, and it is only from the blood of convalescent donors that an active gamma globulin may perhaps be obtained. Mumps convalescent gamma globulin has been used in the treatment of early mumps in adults to diminish complications;[18] convalescent german measles gamma globulin has been used to protect women exposed in the early stages of pregnancy when an attack of the disease may produce severe congenital abnormalities in the infant.[19] The collection and preservation of these antibodies, which only are found in human blood in sufficient quantities during convalescence from certain infections, should be a responsibility of any blood program which is conceived in the public interest.

Hyperimmune gamma globulin has only been prepared from the blood of adults who had previously had whooping cough and were given booster doses of pertussis vaccine. It is valuable in the passive protection of infants and for the treatment of severe cases of whooping cough.[20] Hyperimmunization of humans against other diseases, for example, tetanus or diphtheria, in order to prepare other hyperimmune gamma globins for clinical use seems worth more extensive exploration than it has yet had.

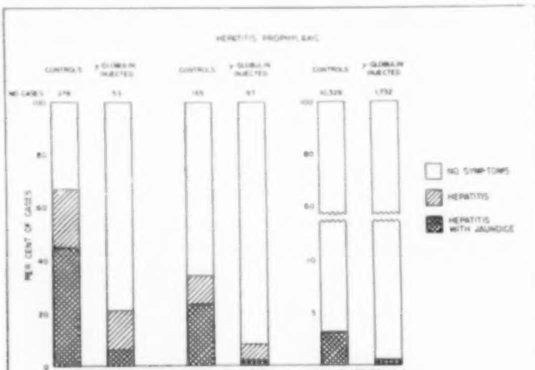


FIG. 4. Summary of results with gamma globulin in the prophylaxis of infectious hepatitis in exposed individuals in three separate outbreaks. (Reproduced from Advances in Internal Medicine, Vol. III, p. 331 with permission of author and publisher.)

Recently, Stokes has pointed out that active-passive immunization (administration of antibody after exposure to an active infectious agent) is an excellent method for inducing an active immunity with mild or inapparent clinical disease. Widely used by veterinarians, it is the method employed when gamma globulin is used to modify measles in an exposed susceptible child. He and his associates have shown that individuals given gamma globulin during an outbreak of infectious hepatitis in an institution may develop an immunity which far outlasts the duration of passive protection from gamma globulin, even though they may exhibit no overt evidences of infection.[21] They call this passive-active immunization and compare it to the natural phenomenon which occurs in infancy when infections such as measles may be mild or unrecognized if exposure occurs during the period when passive immunity from the mother is waning. These concepts extend the possible uses of gamma globulin to simulate the natural processes of active immunization —a combination of passive immunization with exposure to infectious agents in the usual manner.

Recently, specific deficiency of gamma globulin, possibly on a congenital basis, has been recognized and described by Burton.[22] These children are very susceptible to receiving septic infections, cannot produce antibodies in response to antigenic stimuli, but can be protected by regular injections of a moderate dose of gamma globulin every few weeks.

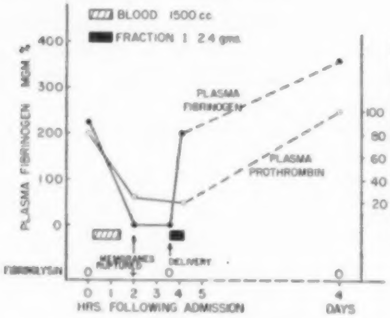
COAGULATION COMPONENTS

Although they comprise a number of different proteins of varying properties and functions, the tendency to interact and lability of the coagulation components has hampered progress in their separation and purification. Products of the new methods have not been available for trial as yet, but should have considerable clinical application, since deficiencies of almost all the major components of the clotting system have been observed and are usually associated with hemorrhagic phenomena, so that rapid methods for restoring blood coagulability are needed. Only three of these components have been prepared in quantity for clinical use by the alcohol-water method: fibrinogen, anti-hemophilic globulin, and thrombin.

Fibrinogen has generally been used as Fraction I, of which it comprises 60-70 per cent of the protein, the remainder containing antihemophilic globulin. Fraction I, which must be stored in the dry state and reconstituted just before use, has had three main applications. It was the original agent added to blood to accelerate formation of rouleaux by the red cells and thus their rapid sedimentation, permitting quick separation of red cells and plasma.[23] It has been used for replacement therapy in two types of fibrinogen deficiency—congenital afibrinogenemia[24] and acquired afibrinogenemia. The latter condition may develop in patients with massive hemorrhage or extensive oozing, particularly if there is any hepatic insufficiency, and has been observed in women with severe intra- or post-partum hemorrhage or with death of an erythroblastotic fetus *in utero*. In these cases, the injection of large amounts (several grams) of Fraction I, providing an amount of fibrinogen which could not possibly be given as blood or plasma, promptly stops the hemorrhage, and is dramatically life-saving (figure 5).[25]

Antihemophilic globulin received very successful trial as a means of bringing the coagulation time of hemophilic blood down toward the normal range during episodes of

hemorrhage or as preparation for surgical or dental procedures (figure 6). Actually, Fraction I, freshly reconstituted, was used for this purpose.[26] This protein is very unstable so that blood loses its potency rapidly on standing. Accordingly, little satisfactory Fraction I has been produced for this purpose, except in the state of Michigan where it has been prepared from very fresh blood. Hemophilic patients in Michigan carry Fraction I with them in case of emergency, so that, if a hemorrhage begins, they can go to a physician and have him administer it to them.



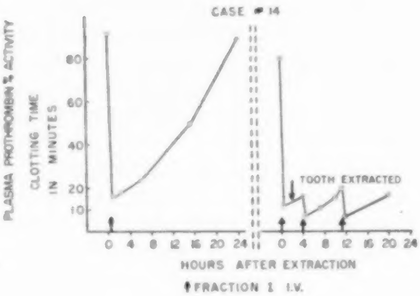


FIG. 5. Severe obstetrical hemorrhage in a women with acquired afibrinogenemia associated with fibrinolysin in the blood. Note failure of 1500 cc. of blood to maintain fibrinogen level, but immediate rise in blood fibrinogen following infusion of 2.4 Gm. of Fraction I. Hemorrhage stopped following the infusion. (This chart from Am. J. Obst. & Gynec. *20*: 379, 1950 with permission of authors and publishers.)

FIG. 6. Response of clotting time of child with hemophilia to intravenous injection of Fraction I. After test injection, shown on left, child was admitted for dental extraction which was carried out without difficulty by first bringing clotting time to normal with Fraction I and by keeping it down with frequent injections. (This chart was made available through the courtesy of Drs. Louis K. Diamond and Wayne H. Borges, Children's Hospital, Boston.)

Thrombin, rather than prothrombin, because of instability of the latter, has been prepared in large amounts as the natural substance most suitable for a local hemostatic agent. To be effective, it must be applied as a solution on some sort of sponge held in place with gentle pressure.[27] Various types of absorbable sponges which can be left in place have been developed— for example, fibrin foam and gelatin foam. Fibrin film, like fibrin foam made from purified thrombin and fibrinogen, has largely been supplanted by films of plastic.

Further exploration of the problem of separation and purification of the various proteins concerned in clotting is urgently needed now that the newer methods of collection and processing point the way. Many clinical situations exist where specific components might be very valuable therapeutically if they were only available for therapeutic use.

HOMOLOGOUS SERUM HEPATITIS

Allusion has been made frequently to the safety of these products. There is extensive evidence that serum albumin and gamma globulin have scarcely, if ever, produced hepa-

titis in recipients, although they have been widely used and are prepared from very large plasma pools.[4a] Unfortunately, this is not altogether true of the coagulation components. Fraction I has been shown to contain hepatitis virus in a fair percentage of lots,[4b] so that two methods of sterilization for this fraction—ultraviolet irradiation and treatment with 2 per cent nitrogen mustard—have been tried with apparent success, but more extensive documentation is needed (table I). Recently, a few preparations of thrombin definitely were shown to be contaminated.[28] However, it seems likely that placental thromboplastin, used for conversion of prothrombin to thrombin, may have been at fault rather than the thrombin itself.

TABLE I

Incidence of Homologous Serum Hepatitis in Patients given Plasma Fractionation Products[1]
Approximate Incidence of Infectivity in Blood Donors 0.2%[1]
Average Incidence of Hepatitis in Recipients of Infected Plasma 37%[1]

Product	No. of Patients	No. of Cases of Hepatitis Expected[1]	Observed
Serum Albumin	33	12	0
Serum Albumin with Plasma or Blood	91	36	2[2]
Gamma Globulin	1977	729	1[3]
Fraction I	47	17	8[4]

[1] The data on which the calculations in this table are based were obtained from a review of the literature (see Paine and Janeway [4a]).

[2] The number of cases observed are the number expected from the blood and plasma administered. 34 cases would have been expected if the albumin alone had been infected.

[3] This one case was probably an instance of infectious hepatitis. No other among seventy children who received this lot of gamma globulin developed hepatitis. ([4b])

[4] From figures reported to American Red Cross from use of Fraction I in hemophilia. The incidence among a few individuals receiving Fraction I for afibrinogenemia has been very high; no cases have been reported since treatment with 2% nitrogen mustard was instituted as part of the process of preparation, but a careful follow-up study has not yet been made.

SUMMARY

Serum albumin and gamma globulin, prepared by the standard alcohol-water method of fractionation, have found a permanent and important place in the treatment and prevention of disease. Their convenience, availability, safety, and standardized potency make them ideal therapeutic agents from the standpoint of the physician or public health worker and have permitted their widespread use in a series of clinical investigations which have extended our knowledge of human physiology and its disturbances in disease.

Fraction I, as a source growth of fibrinogen and of antihemophilic globulin, has demonstrated its great clinical value in the control of hemorrhage due to afibrinogenemia or hemophilia but cannot be considered an ideal product due to difficulties with the stability of antihemophilic globulin and to frequent contamination of these lots with hepatitis virus. Thrombin is readily prepared as a valuable natural hemostatic agent for local use in combination with any type of absorbable sponge.

The new methods of blood collection and processing described in the preceding paper offer an inviting prospect for clinical progress, since they should lead to far better preservation and separation of the labile coagulation components, for which specific clinical

indications are obvious. The possibility of collecting, and preserving for use when needed, gamma globulins from individuals convalescent from certain infectious diseases and gamma globulins from hyperimmunized humans, by methods which permit conservation of other valuable blood components from the same donations, such as albumin, fibrinogen and red cells, deserves more attention than it has had to date.

It is my personal belief that we are passing through a revolution in blood technology, to which laboratories such as this will continue to make important contributions, as a result of which the physician will ultimately have at his command specific, standardized, safe therapeutic agents preserved in their natural state, for use when needed to correct the physiologic disturbances of as many deficiencies of specific blood components as current medical knowledge permits him to recognize. This list is constantly growing. For example, during this current year, as a result of research technics which are the direct outgrowth of the development of plasma fractionation, our own laboratory has recognized one specific plasma protein deficiency[29] and confirmed the existence of another,[30] both of which were unknown a year ago.

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Studies of the Effects of Modified Human Globin in Man

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Since more than 2500 units of modified human globin have been administered to between 500 and 1000 patients, by 20 or more groups of investigators, it is time to compile a report on the clinical experience with this material as a source of parenteral protein and as a plasma extender.

The first modified globin for human use was prepared by Dr. Max M. Strumia of the Bryn Mawr Hospital, who has also done much of the work on the subject.[1, 2] Most of the globin used in the studies summarized here was made by Sharp & Dohme, Inc.,[3] essentially using Dr. Strumia's method. The reports are being gathered together through the kindness and with the permission of the many investigators concerned. Although globin is a promising agent, it will become apparent in the report to follow that more work must be done to establish the place of globin in our therapeutic armamentarium.

A saving of large quantities of human blood would result if, in fact, globin is as safe and useful as reported by most investigators using it. Consider that the usual donation of a pint of blood furnishes a maximum of 15 Gm. of plasma protein, while the red cells, after processing, will yield over 30 Gm. of globin, or about twice as much in terms of grams of protein. The Red Cross is now collecting and processing to plasma, blood at the rate of about 3 million units per year.[4] The red cells are not used now, even for pig feed. From these red cell residues at least 100,000 Kg. of globin could be produced. This would represent about six million 16 Gm. units of globin, the size in which it is customarily used. If this quantity of globin were used for parenteral feeding, it would be sufficient protein to maintain 100,000 persons for 10 days at an intake of 100 Gm. of protein daily. However, the effort expended in saving this quantity would be wasted were the material not good for something. In the following paragraphs, the evidence that globin, administered parenterally, is metabolized, may be used for protein building, and has a degree of effectiveness as a plasma expander, will be presented.

Modified human globin is prepared[5] from hemolyzed sterile human erythrocytes by precipitation from acid acetone. The crude globin so formed is then digested in an alkaline medium for 27 hours at 37 C., is dialyzed, clarified, aged at 37 C. for two weeks, bottled, and lyophilized. Stringent sterility, pyrogen, and safety tests are then conducted before clinical study is begun. The dried powder is colorless or slightly yellow, readily soluble in normal saline solution, and more slowly soluble in glucose and distilled water. About 16

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per cent of globin is nitrogen, only 0.25 per cent being free amino nitrogen. As presently prepared, the powder contains about 5 per cent sodium chloride, no detectable potassium, from zero to 3.0 mg. of iron per 100 Gm., about 0.18 mg. phosphorus per 100 Gm., and from 9 to 18 mg. calcium per 100 Gm. About 80 per cent of the protein has a molecular weight of approximately 34,000 and nearly 90 per cent travels as a single component during electrophoresis, migrating at a rate between that of the alpha and $alpha_1$ globulins. In one study approximately 35 per cent of the material passed through the membrane during prolonged ultrafiltration. The isoelectric point is about pH 5.5. Pennell has said,[5] "that at least 65 per cent of the modified globin is fairly homogeneous and behaves as a gobulin by precipitation tests. Of the remainder, a large part has the same electrophoretic mobility as does the bulk of the material. An appreciable part of the heterologous material must also be very similar in size to the bulk of the globin, as judged by ultracentrifugation." Cohn[6] states that globin (modified) "has a diameter more nearly comparable to the dimensions of the plasma proteins than those of morst other suggested blood substitutes."

Globin has been administered intravenously and subcutaneously usually as a 4 or 8 per cent solution made up in either isotonic saline or 5 per cent dextrose in water. Saline is recommended for most purposes, since, as indicated above, globin is more rapidly soluble in this solution. Early studies were done with globin dissolved in distilled water, but it was soon observed that hemolysis was produced *in vitro*.[7] Sufficient glucose or electrolyte must be present to make the resultant solution isotonic.

Reports of reactions to the intravenous infusions of globin have varied greatly from clinic to clinic. Chiefly, two kinds of reactions have been observed. The first, and most frequent, is characterized by flushing, itching, and occasionally urticaria. This reaction appears to be related to the rate of administration and perhaps to the total quantity of globin administered. It disappears quickly when the infusion rate is slowed. With early lots of the material, this reaction occurred in 35 out of 52 infusions in one group of studies,[7] but with more careful preparation and testing before release the reported incidence has decreased greatly, so that now it is seldom noted.[8-11] Pyrogenic reactions were frequent in a few of the early lots, but recently with the more stringent testing this reaction also has been seen less often.[8-11] No consistent or significant changes in blood pressure, pulse, or respiration have been observed where globin has been given to individuals not in shock. Nausea and vomiting have been reported after four to six days of globin infusions in a few instances.[12] The explanation for this reaction is not apparent. The incidence of all reactions observed by the more than 20 groups of investigators in greater than 2500 infusions has been 2.7 per cent.

When globin is administered intravenously, some is excreted in the urine,* the amount found varying greatly in the reports from different investigators. Two groups have found that approximately 25 per cent of the administered protein was excreted daily,[7, 12] while most other investigators have found the excretion to be much less, usually from 1 to 5 per cent, occasionally as high as 15 per cent.[13-15] It has been suggested that some of the protein found in the urine is not globin, but albumin which, if true, might imply renal damage from globin administration. Such a state of affairs seems unlikely, however. First, one

* Method described by F. E. Kendall and L. L. Abell, unpublished.

group of investigators has examined the urine [carefully and repeatedly] in patients receiving [multiple] globin infusions and has found no abnormality except the presence of a protein precipitated by Esbach's reagent but unprecipitated with heat, characteristic of globin but no albumin.[7] Second, the proteinuria does not persist long after the infusion—usually none being found the following day[7, 13] Finally, renal damage has not been found, although it may have occurred in one instance.[16] Thus, the bulk of the evidence at present indicates that most, if not all, of the protein in the urine is globin, and that the globinuria

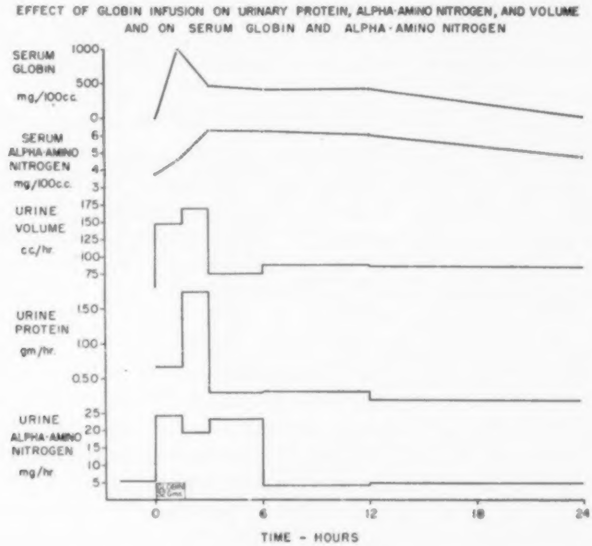

FIG. 1

is not damaging to the kidneys. More work of greater precision on these matters is called for. One further point concerning renal excretion may be made. Globin *in vitro* gives a positive guaiac and benzidine test, as does the protein in the urine after infusion.[7] Thus, when the urine postinfusion gives positive reaction with these tests for blood, hemoglobinuria and hematuria may not have occurred, but simply globinuria. The hemoglobin degradation product accounting for this positive test has not been identified. One instance of probable hematuria or hemoglobinuria has been reported.[17]

Following its infusion, globin disappears from the blood,* usually within 24 hours.[7, 18] For example, following the infusion of 32 Gm. in one and one-half hours, the serum globin reaches a peak of 1000 to 1500 mg. per 100 cc., then rapidly falls to about 500 mg. per 100 cc., where it may remain for about 12 hours and finally disappears in the succeeding 12 hours (figure 1). Twenty-four hours after the last infusion of 32 Gm. of globin daily for six days to 1 patient, globin could not be detected by electrophoresis.[7] This disappearance is in part accounted for by renal excretion, the remainder probably being metabolized or converted.[7, 13]

* Method described by F. E. Kendall and L. L. Abell, unpublished.

TABLE I
Amino Acid Analysis of Modified Human Globin

Essential Amino Acids Determined	Modified Human Globin 286-166-I and 286-180-A
Methionine	1.1%
Tryptophane	1.6%
Isoleucine	0.62%
Leucine	14.6%
Valine	10.9%
Histidine	8.4%
Arginine	3.5%
Threonine	3.8%
Phenylalanine	5.7%
Lysine	11.9%

Several studies support the concept that parenterally administered globin is metabolized in man and the dog.[19] First, it is a protein naturally found in the body and as such might be expected to be metabolically available. Second, balance studies in undernourished individuals show positive nitrogen balance.[7, 12, 20] Third, in osteoporosis, infusions of globin have been accompanied by positive nitrogen balance and a decrease in urinary calcium and phosphorus, suggesting increased bone formation.[13, 20] Fourth, retention of potassium occurs, presumably indicating incorporation of globin into protoplasm.[13] Finally, globin usually disappears from the blood within 24 hours after even repeated infusions.[7, 18]

In contrast to globin infusions, the daily administration of concentrated human serum albumin is followed by a progressive rise in the plasma albumin concentration.[21, 22] Following cessation of albumin administration, there is a slow return of the albumin concentration to its previous level so that such injected albumin has an approximate five to ten day half-life.[22, 23] From these and similar studies, it seems clear that not only is globin excreted in the urine in much larger quantities following infusion than is albumin but also that the disappearance of globin from the blood is much more rapid.

As pointed out above, globin will maintain a positive nitrogen balance in an undernourished individual. This occurs even when globin furnishes the sole source of dietary nitrogen, either parenterally or orally administered.[7] The amino acid content[5, 7] of hydrolyzed human globin measured microbiologically (table I) would suggest that isoleucine might be a limiting amino acid. However, the positive nitrogen balances occurred in spite of the low isoleucine content of the material given orally and were not augmented by supplementation with this amino acid.

The total plasma protein and plasma albumin concentrations have often been observed to rise following repeated globin infusions, especially in patients with hypoalbuminemia.[14, 24-27] This rise has been observed in patients with various kinds of carcinoma, in the nephrotic syndrome, in simple undernutrition, but, as the one exception, not in patients with cirrhosis of the liver. These data have suggested the possibility that globin may be converted rather rapidly to albumin, if so presumably in the liver. Moreover, following intravenous globin infusion there is a rise in serum polypeptide and alpha amino nitrogen and also in their urinary excretion. Since these increases are out of proportion to the quantity of polypeptide and alpha amino nitrogen contained in the globin infused, their presence supports the concept that globin is broken down to polypeptide and amino

acids and is then resynthesized, possibly into plasma albumin by the liver.

When modified human globin is given intravenously in the absence of shock, there usually is a fall in the blood hematocrit. The rate of fall and the duration reported have varied between investigators. For example, in one study the rate and duration of the hemodilution as measured by hematocrit fall was similar to that seen following an equal volume of normal saline,[7] while others did not observe the maximum drop until 6 to 12 hours after infusion.[28] These latter results with globin are also at variance with those found after the administration of concentrated human albumin in individuals with normal blood volumes. In this situation the hemoglobin concentration reaches its minimum before the end of an hour after the infusion and comes gradually back to its initial level by 8 to 10 hours as a rule.[21] These differences are at present not explicable.

The plasma volume (measured by Evans' blue dye) usually increases significantly when globin is administered intravenously to patients not in shock.[26-28] When infusions are given daily for several days, the expanded volume often persists for 24 hours or more.[29] In [the] few patients undergoing operative procedures, cardiac output was increased as measured by the "Ballistic Index."[30]

Previously hydrated normal individuals or convalescents given globin intravenously showed, according to the results of one study,[31] no significant change in renal plasma flow, or urine flow, although there were small increases in sodium excretion rate. Other investigators have observed a variable diuresis, usually much greater than that following infusion of an equivalent amount of saline.[7] The differences between these results with respect to urine volume and flow may be the state of hydration of the patients before infusion.

Marked increases in sodium excretion have been observed after globin infusion to patients with cirrhosis of the liver, especially those with ascites.[32] In the nephrotic syndrome, many patients have a satisfactory diuresis, much the same as that following albumin administration, although perhaps somewhat slower.[14, 17, 24, 33] In some instances, the serum protein increased, but this was not necessarily parallel to the diuresis which occurred. In 10 patients with [a] the nephrotic syndrome, 9 had a good diuresis following 32 to 64 Gm. of globin daily for five days.[34] Thus, whatever the physiologic mechanism, globin given intravenously may prove to be of therapeutic value in patients with fluid accumulations associated with hepatic cirrhosis or nephrosis and perhaps in other states as well.

The action of globin as a plasma extender has been evaluated in a number of patients with severe burns and with peripheral vascular collapse from other causes. Such studies are always difficult to interpret because of the unpredictability of the course of these very ill people and because it is seldom possible to administer one agent alone. The investigations in normal individuals of hematocrit and plasma volume changes suggest that globin may be a fair plasma extender. The evidence, especially from studies using I^{131}-tagged globin,[18] indicates that such substances as dextran, polyvinylpyrrolidone, and albumin give greater and more prompt effect than globin, and may exert their effect over a longer period of time. Because of its low molecular weight, as compared to albumin, the rapidity with which globin leaves the blood stream, its relatively rapid metabolism, and significant urinary excretion, it might be expected that globin would be a poorer plasma expander than albumin or the other substances listed above. Nevertheless, the results to date suggest

that globin may be satisfactory for this purpose in shock and in burns.[1, 10, 17, 27, 28, 35, 36] The advantage of using a naturally occurring substance and one which is fairly rapidly metabolized are obvious and do away with the objections which may be raised against dextran which is relatively slowly metabolized, and polyvinylpyrrolidone which appears not to be metabolized at all, and is very slowly excreted after the first few hours. Thus, if repeated injections of a plasma extender are necessary, it may be that globin will find an important place with perhaps not as dramatic effect as that of albumin, but one which will supply protein for metabolic needs much more rapidly. For example, 1 patient received approximately 7300 cc. of globin in the first 36 hours after hospitalization following a severe burn. It was a clinical impression of the investigators that "the patient received the same benefit from globin that he would have received had he been given plasma."[36]

In all, approximately 60 cases of shock and burns have been studied. Certainly, many more studies are necessary, both in animals, if applicable, and in man. However, the impression is that globin may compare favorably with other substances as a plasma extender and possesses some inherent advantages.

There has been some criticism of the new plasma extenders because, in common with many macromolecular substances, they cause rapid sedimentation of red cells, rouleaux formation, and difficulty in typing and crossmatching. In addition, it has been suggested that they might cause *in vivo* "sludging," particularly in shock, which would lead to a poorer circulation of red cells than would be expected from the increase in plasma volume. These criticisms so far have not been raised against globin. It does not cause a sharp increase in sedimentation rate, following intravenous administration, nor is any difficulty encountered in typing or crossmatching. To date, a search for "sludging" has not been made in shocked animals or in man, but considering the present knowledge of globin, it is doubtful that such would occur.

The risk of serum hepatitis following globin administration seems hardly to exist. The process of preparation (e.g., incubated at pH 11 for 27 hours) would seem to preclude the survival of viruses, no matter how hardy. Furthermore, no instances of serum hepatitis have been reported following the injections made so far. Finally, when deliberately added to red cells, hardy viruses such as the SK poliomyelitis and the Eastern equine encephalitis were not detectable in the globin subsequently produced.[5]

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The author wishes to express his appreciation to the many investigators who generously permitted their work to be quoted in this summary. Particular thanks are due Doctors William P. Boger and Archer P. Crosley, Jr. of Sharp and Dohme for their continued enthusiasm and their labors in gathering together much of the data presented here.

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The Clinical Status of Dextran, PVP and Gelatin Products

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The terms—plasma expander, plasma volume expander, and plasma substitute—have come into being recently to denote a class of solutions of varied chemical composition but having a common property, namely, the ability to support the circulation in the shock state. Circumstances often necessitate the use of less than ideal measures in the treatment of large numbers of casualties in a disaster. Experiences in World War II have established the unique position of whole blood in the treatment of wound shock. Plasma also was important in the saving of many lives, but abundant clinical observations attest to its shortcomings in resuscitation of the seriously wounded.

The difficulties with these biologic materials, especially blood, lie in a number of important considerations: (1) the amounts procurable in relation to estimated needs; (2) the problems of storage, including the need for refrigeration, and deterioration on aging; (3) the necessity for typing and crossmatching the material to the individual patient; (4) the danger of transmitting the virus of hepatitis.

Even though the circulating volume of blood and plasma is maintained after injury with hemorrhage, failure to supply the vital organs with sufficient oxygen may lead to irreversible organ damage. Further administration of fluids, or even whole blood, may then be ineffective in resuscitating the patient. Available whole blood in a major disaster should, therefore, be used, as far as circumstances permit, for casualties with the most severe injuries. When loss of blood has not been excessive, it is permissible and much more economical to use a blood derivative or one of the plasma volume expanders.

The fluids used when whole blood is not available may be classified as blood derivatives and plasma expanders. The blood derivatives are plasma, serum albumin, and globin. The latter is now undergoing rapid development and intensive study. Plasma aids in maintaining and stabilizing the blood volume, transports red blood cells, antibodies, nutrients, and hormones to the tissues, and provides components for the clotting mechanism. The most serious complication resulting from the use of plasma has been homologous serum jaundice. One problem urgently requiring solution is the development of a method of sterilization of pooled plasma, which will destroy the virus and prevent the transmission of disease. Although albumin, produced by the fractionation of plasma, has certain advan-

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tages and disadvantages over plasma, the problems of sterility and supply are the same as for plasma and, in addition, the product is expensive and available only in very limited amounts. Five units of plasma are required to produce one unit of albumin. Bovine albumin was tested during World War II, but, because it could not be made nonallergenic, its use was discontinued. Despeciation of animal plasma is currently being investigated.

The use of a chemically modified human globin preparation as a plasma substitute was first described in 1945 by Strumia et al, who reported that processed human globin solutions showed promise as safe and effective preparations in replacing lost blood in patients suffering from severe shock. Globin is prepared from unwashed red blood cells salvaged in the processing of plasma and plasma derivatives. The resulting product remains stable for a period of years, either in the dried form or in solution. Between two and three units of globin, each containing 16 Gm. of globin and osmotically equivalent to 250 cc. of plasma, may be obtained from the cell residue of a single bleeding. As yet it cannot be administered with complete freedom from reactions. A fraction of the amount infused is excreted in the urine. The fate of the remainder is now undergoing study. These globin solutions show some promise as plasma expanders. Other chemical modifications have been applied to globin but the products are still in the experimental stage.

Because of the difficulties in building up stockpiles of whole blood and blood derivatives for a national emergency, there is widespread interest in a group of storable substances which cause and maintain an increase in the circulating blood volume, namely, the plasma expanders. Many such substances have been described and studied, particularly those of large molecular size. Gum acacia in six per cent solution was used in World War I and thereafter until it was learned that the gum was stored in the body indefinitely and produced tissue changes in such organs as the liver. Pectin, a polysaccharide derived from citrus fruits, has been tested and its use discontinued because the product could not be standardized. In the past few years new plasma expanders have been investigated, including gelatin and its derivatives, dextran, and PVP (polyvinyl pyrrolidone). Each has its merits and its deficiencies. The following characteristics should be found in the ideal plasma substitute: (1) osmotically, it must be the equal of plasma; (2) its viscosity and retention in the blood stream must be about that of plasma; (3) it must be stable on storage; (4) it must be nonantigenic; (5) it must be nontoxic; (6) it should have no unfavorable effects on cell structure and function. The gelatin products, dextran and PVP, only partially meet these requirements.

DEXTRAN

The dextrans make up an ill-defined class of macromolecular carbohydrates produced by a specific group of bacteria—mostly of the genus *Leuconostoc*—acting on sucrose. As contaminants in sucrose refining and storage, they form a troublesome, viscous slime. On the basis of his work on these problems, Ingelman chose the material as a possible plasma expander.

The native dextrans are similar in that they are made up of glucopyranose units with alpha 1, 6 linkages predominant. Alpha 1, 4 linkages, thought to be associated with branching of the chain, are present in widely varying frequencies (from ⅓ to 1/20 of the total links) in different cultures, the primary determinant of the proportion apparently

being the active bacteria or enzymes. It also may be modified somewhat by various growth supplements in the culture medium. For use as a plasma expander, the very large native molecules having molecular weights of several million are partially broken down, usually by acid hydrolysis, to form units of a size found to be most effective (molecular weight of about 75,000). Clinical dextran, like all the plasma expanders, consists of molecules of widely differing sizes. The information derived from osmotic pressure measurements, the number average (Mn), is not of great physiologic use because osmometer membranes retain particles which pass rather freely through capillary walls. The *in vitro* and *in vivo* colloid osmotic pressures may, therefore, be quite different. Light scattering measurements, supplemented by measurements of viscosity, provide data for the calculation of the weight average molecular weight (Mw) which is of use in drawing up production specifications.

Following the intravenous administration of the available clinical preparations, dextran is excreted primarily in the urine. Only minutiae appear in the normal gastro-intestinal tract. In the presence of disease, this may not be so. Large quantities (100 Gm. in 24 hours) of dextran have been reported in the gastric juice of a man with fatal uremia and ileus of 12 days' duration. The rate of urinary excretion is reported to be between 10 and 40 per cent in the first 24 hours, variations depending on characteristics of the material as well as the state of the animal. There is a slow metabolic breakdown of the material to CO^2 in the tissues; and at the end of 72 hours, dogs given C^{14} marked dextran were found to have 65 to 70 per cent of the radioactivity in the urine, four to six per cent in expired CO_2, one-half per cent in extracellular fluids (including plasma), three to five per cent in the viscera, and about 25 per cent unaccounted for. That reticulo-endothelial or other storage does take place is indicated by the finding of C^{14} labeled material in liver, spleen, and lymph nodes. Microscopic evidence of damage has not been reported in these organs. Studies of the fate of C^{14} labeled dextran in human subjects have shown that, two to four weeks after administration, essentially all of the dose can be accounted for. The smaller molecules are excreted unchanged in the urine while the larger ones are slowly metabolized. Some of the carbon circulates for a while in the general metabolic carbohydrate pool but is eventually released and excreted as CO_2.

A characteristic toxic reaction of allergic nature in humans has been reported with some dextran preparations. Various manifestations include flushing, urticaria, angioneurotic edema, wheezing, vasomotor instability, generalized aches and pains, and delayed painful, joint swelling. It is presumed to be an immunologic reaction and has been reported by American groups using the Swedish preparation "Macrodex." It was encountered in approximately 40 per cent of military subjects receiving 500 cc. of less of a six per cent solution. It should be noted that such a high reaction rate has not been reported in Sweden where the same material was used. Reactions were trivial or absent in military patients under anesthesia. Under the same testing conditions with American preparations of dextran, the reaction rate was also very low.

The potentiality of dextran's producing allergic reactions in man had been indicated some years ago by Hehre and Sugg who demonstrated that certain native dextrans shared antigens with several *pneumococci* and that guinea pigs sensitized with pneumococcal antiserum could be fatally shocked with dextran. More recently, Kabat has found that native and clinical dextrans, if given subcutaneously, will behave as antigens and induce

antibody production and cutaneous reactivity in humans. Antigenic experience with *Leuconostoc* and its related haptenes appears to be fairly common in the American population; approximately, 20 per cent of normal adults have recently been found to possess agglutinins to this organism. On the other hand, attempts to correlate immunologic reactions with laboratory and clinical tests for the presence of sensitivity have been unsuccessful. Reactions to intravenously administered dextran have thus far failed to correlate entirely with either skin sensitivity or anti-*Leuconostoc* antibody, and it is not possible at the present time with the methods available to predict in what manner an individual will react to an infusion.

Tolerance to the material is not developed. A subject who reacts to certain preparations will continue to react on repeated administration. It has been demonstrated, however, that a normal subject may lose his reactiveness under anesthesia.

Specific tissue or organ impairment has not been encountered to a significant degree in the extensive clinical use of dextran, but there are some observations which require further elucidation. Two investigators have reported a significantly reduced renal para-aminohippuric acid transfer maximum, which persisted for at least a week in one of two dogs and in five of nine normal humans with antecedent renal impairment, following infusion of large amounts of dextran over a period of several days.

In a study of the molecular weight of dextran, it has been found that the renal excretory threshold for molecules is slightly larger than 36,000 molecular weight. The problem of molecular dimensions and shape is, however, complicated by the presence of variable amounts of branching in the molecular structure which demonstrably affects its physical and chemical behavior. Studies of this factor are being undertaken, but definitive results are not yet available.

Reports of the clinical use of dextrans in research hospitals in this country are rapidly accumulating. The Emory University group has studied the restoration of normal circulatory dynamics in control subjects following the substitution of dextran for an equal amount of blood. Continuing depression of the hematocrit for several hours in these cases is evidence that the dextran largely remained in the vascular system. This conclusion is further supported by the restoration of pulmonary arterial pressure and cardiac output. Fifteen patients in shock received dextran, usually with satisfactory improvement of pulse and blood pressure.

Dextran is now being manufactured and distributed as an experimental drug by several firms in the United States, and it will undoubtedly form a considerable portion of the national emergency stockpile. While its chemical composition and physical characteristics are subject to modification in the light of rapidly accumulating research information, it appears certain to occupy an important role among the plasma expanders. It may be possible to develop this material so that it can be administered in the field by medical aides and without the need for warming to body temperature, with reasonable safety and with likelihood of definite osmotic advantage to the patient.

PVP

Polyvinyl pyrrolidone, or PVP, is a synthetic polymeric macromolecule, which was first produced in Germany and selected by Weese as a potential plasma substitute follow-

ing his investigation of many possible substances. It is an entirely chemical synthetic material now recognized as potentially useful; since the processes involved allow truly mass production, it is likely that, in spite of certain serious shortcomings, it may form a large part of any plasma expander program which might be undertaken in the near future. Under the names "Kollidon" and "Periston" large amounts were produced for use in the German army and civilian population. In spite of several continental reports of its successful and nontoxic administration in treatment of shock, a special American team, sent to Germany in 1951, was unable to obtain enough trustworthy records of such administration to allow any specific conclusions; no other data pertaining to the late effects of the material have been found. It should be noted that, since the war, the use of PVP solutions has not been widespread in Germany, preference having been given to plasma, which is now readily available. Since the war, PVP, under the name of "Subtosan" has received increasing attention in the French literature; at present its use is allegedly widespread in that country. In England a PVP preparation called "Plasmosan" has been tested on animals and on patients with no signs of toxicity, although as much as 3.5 liters were given to 1 patient in one week. Even larger amounts, up to 20 liters, have been given to single subjects in this country without immediately demonstrable ill effects, but such quantities are at present definitely not advisable.

The physicochemical characterization of the molecule is being pursued vigorously, but as yet no clear-cut picture of its shape or dimensions is possible. Empirical relationships have been established between viscosity, which is fairly easy to measure, and molecular weight of PVP solutions. This forms the groundwork for practical studies of the distribution of molecular sizes in any preparation and the relation of size to bodily diffusion and excretion.

The material in the dry form is stable on storage and is nonantigenic. There is extensive evidence that its effectiveness is approximately equal to that of plasma in restoring diminished blood volume resulting from hemorrhage. However, the duration of action, as discussed later, is probably variable, but potentially controllable within unknown limits by proper regulation of the distribution of molecular sizes.

The ultimate fate of PVP in the body is undergoing extensive investigation. Following infusion, from 40 to 60 per cent of the material now available can be accounted for, apparently unchanged in the urine in the first 24 hours. With C^{14} labeled PVP, it has been found that insignificant traces of the constituent carbon appear as exhaled CO_2. Following administration of PVP to adults, microscopically visible storage of PVP in reticulo-endothelial cells has been reported. This has been substantiated in animals and is probably true of man. Such storage in infants has, however, been reported several times, and one worker feels that there may be an inflammatory tissue response and interstitial pneumonia secondary to such storage. The latter finding is contrary to general experience and the attributing of these changes to PVP is not widely accepted.

Excretion studies and especially distribution and excretion studies using tracer PVP labeled with radioactive —I^{131} and C^{14} have demonstrated conclusively that storage does occur with K values above 31,000, even with small doses. This fact must be acknowledged in the use of any presently available preparation. A decision as to the presence of chronic toxicity is not yet possible.

GELATIN AND ITS DERIVATIVES

Gelatin is the soluble, denatured, protein product produced from animal collagen principally from beef bones, calf, and pork skins. In its production, the large insoluble collagen molecules are broken into fragments of quite uniform size. In its adaptation for use as a plasma substitute, these molecules are further broken down by various means; but in this step the homogeneity of size is lost, and the resulting material contains a wide spectrum of molecular weights. Little control, either in production or refining, has been exerted over this distribution. The means used for the final breakdown influences the characteristics of the resultant material; by control of the degradation, efforts are being made to produce a large group of products from which the most satisfactory product may be chosen. The commercial gelatins designated P-20 and P-180 are produced by autoclaving for periods of 20 and 180 minutes. The products have gel points of 27C. to 30C. and 12C. to 15C. (6 per cent solutions) and mean molecular weights of about 33,000 and 19,000, respectively. The usefulness of the P-180 gelatin is slight because of an excessively rapid renal excretion and, presumably, transcapillary equilibration. Condensation of gelatin (polymerization by the action of glyoxal), followed by oxidation with hydrogen peroxide, results in "oxypolygeltatin" with a mean molecular size of about 33,000 and a gel point of 10C. to 13C. (6 per cent solution). Recently, gelatin has been modified in other ways by treatment with an aromatic amine producing "fluid gelatin." The gel point of these solutions approaches that of freezing (0 to 4 degrees in 6 per cent solution), which would probably eliminate any objection to its use on that basis.

A preliminary comparison of the action of fluid gelatin with other plasma expanders in dogs and in man subjected to mild hemorrhage or hemorrhagic hypotensive shock has been made. In the former, fluid gelatin compares favorably with the other gelatin; in the latter, it appears to be more effective than either the other gelatins or dextran. The present intensive work on gelatin derivatives gives promise of useful results, but much testing and evaluation of the newer products still remains to be done.

The handling of the gelatin products by the body is the least defined of the three substances under discussion. This is due, in part, to difficulty in obtaining material labeled with radioactive tracers. Within rough limits, the injected substance not found in the urine remains in the plasma. The presence and the amount of metabolic utilization of administered gelatin has yet to be definitely established. The inference that metabolic breakdown of the gelatin is indicated by the finding of increased urinary urea nitrogen and a positive nitrogen balance during periods of gelatin infusion is still a matter of debate. The rate of urinary excretion of all the products so far studied seems to be dependent on the molecular weight and independent of the chemical modifications of the original gelatin molecule. Thus: Of the most highly heat-degraded gelatin, P-180, 75-80 per cent is excreted in 24 hours, while for P-20 the excretion is about 45-55 per cent in the same time. Oxypolygelatin is excreted a little faster than P-20 and fluid gelatin at rates varying greatly but generally resembling those of oxypolygelatin.

There is no information concerning the transcapillary movement of gelatin in shocked patients, but there is no reason to believe that it differs significantly from that of PVP or dextran.

No definite toxic effects of the gelatin derivatives now being investigated have been reported. It appears that the absence of antigenicity has been well established. No significant changes of clinical liver function tests and of the renal function indices have been observed in humans or in animals receiving large doses.

The precise description of the effect of the molecular weight of gelatin on the fate of the material has been barely touched. Because of the lack of undesirable side effects and the difficulty in controlling the physical properties of the solution, which are also dependent on molecular weight, it has appeared sufficient to aim at a mean molecular weight of about 30,000, and no closer control has been attempted.

The difficulty with blood typing and crossmatching which is encountered after infusions of gelatin must be taken into consideration, and it is advisable that samples for bloodmatching be taken prior to the infusion.

DISCUSSION

With all the macromolecular materials so far considered, an ordinary sample of any plasma expander is found to contain a wide spectrum of molecular weights. It has become apparent that knowledge of the relation between molecular weight and biologic handling is essential to the realization of the full potentiality of these substances. Two specific questions have arisen to which answers are being sought.

1. What is the effect of molecular weight on renal excretion? This assumes special importance since it appears well demonstrated that the kidney is the only significant route of excretion. The bile, feces, and expired CO_2 contain only minutes quantities of the materials or its breakdown products. At present the requisite experiments have not been completed, but more data should be forthcoming in the near future. There is good evidence that the rate of excretion falls rapidly as the molecular weight increases and reaches a definite cut-off level in the range of 30,000 to 40,000. Further, the amounts of materials appearing in the urine approximate the estimated portions of injected materials having molecular weights below that cut-off value.

2. At what molecular weight do the materials lose their intravascular osmotic effect? It is reasonable to assume, and it is indicated by the scanty available data, that as the molecular weight decreases, the materials will move across the vascular limiting membranes more rapidly and at some level will become too rapid for clinical effectiveness. Whether loss of effectiveness will occur at a sharp limiting size, or with a gradual fall, is of considerable theoretical as well as practical interest, but to date efforts in this direction have produced little definitive data. The best estimates at the moment indicate that it is within the same range as the glomerular threshold (27,000 to 40,000) or perhaps higher.

CONCLUSIONS

The plasma expanders, in the future, have an important place in medicine and one which promises to become more important as the disasters of both peace and war tend to produce large numbers of casualties in small and isolated areas of devastation. The sound principles of intravenous countershock therapy must never be forgotten. When life is threatened by the loss of whole blood, whole blood must be supplied; for this there is

no substitute. Under limitations of time, personnel, materials, and detailed medical supervision, where it is necessary to make the best of the situation, the plasma expanders will probably be useful if their limitations are appreciated and their undesirable effects are understood and controlled. Meanwhile, it is clearly necessary to study their action in the laboratory and in the clinic in order to gain the knowledge required for their use under the severe life-saving demands of an emergency.

ACKNOWLEDGMENTS

The author is indebted to colleagues in his own laboratory for some of the data used in this paper. Much of the information has been made available by the Division of Medical Sciences of the National Research Council. Many investigators cooperating in the plasma substitutes program have sent frequent reports of current research progress, and access to this material has been an invaluable aid. Their contributions will be recognized as they appear in the scientific journals.

ABSTRACTS

medicine

NEUROLOGY

161. *Primary Cerebral Thrombophlebitis.* J. CHANDLER SMITH, Chicago, Ill. J. A. M. A. *148*:613-23, Feb. 23, 1952.

A case is presented in which a Jewish male of 52 years experienced for 6 years transient episodes of dizziness, headache, and fever terminating in convulsions, flaccid paralysis, and coma. Autopsy disclosed thrombophlebitis of the right frontal and both parietal lobes of the cerebrum. No lesions of cerebral arteries were found and arterial changes identified elsewhere were consistent with arteriosclerosis. The anatomic diagnosis was primary thrombophlebitis of cerebral veins.

Primary thrombophlebitis affects chiefly the superficial veins of the extremities, is recurrent and of migratory character. The course is self-limited and recovery is ordinarily complete. The cause is unknown although phlebosclerosis, defective uric acid metabolism and an unidentified toxin have been suggested. Anatomic examinations of several cases have revealed involvement of veins of the viscera and in these cases a high incidence of pulmonary infarct was noted. Clinical manifestations indicating involvement of cerebral veins include convulsions, loss of consciousness, headache, coma, paresthesias, aphasia and transient pareses. Anatomic descriptions of thrombophlebitis associated with Buerger's disease were not found. So far as can be determined, this constitutes the first record of the clinical course and anatomic examination of a case of primary cerebral thrombophlebitis. 23 references. 2 figures.—*Author's abstract.*

HEMATOPOIETIC

162. *Relationship of Glandular Mucoprotein from Human Gastric Juice to Castle's Intrinsic Antianemic Factor.* GEORGE B. JERZY GLASS, LINN J. BOYD, MICHAEL A. RUBINSTEIN AND CHESTER A. SVIGALS, New York, N. Y. Science, *115*:101-08, February 1952.

Vitamin B_{12} can induce remission in pernicious anemia when administered parenterally, but is far less effective when administered orally. This has stimulated further research into the intrinsic factor that might be present.

The authors have studied the glandular mucoprotein present in human gastric juice and have shown that with the addition of this mucoprotein a small dose of vitamin B_{12} would potentiate action of the vitamin when administered orally.

Nine patients with pernicious anemia, either in relapse or previously untreated, were studied.

The authors indicate and present evidence that it may be permissible to consider glandular mucoprotein in the gastric juice as the main carrier of the intrinsic factor activity. 26 references. 1 table. 1 figure.

RESPIRATORY

163. *Review of Mass Chest X-Ray Surveys.* ROBERT J. ANDERSON, M.D., Washington, D. C. J.A.M.A. *148*:605-08, Feb. 23, 1952.

As the community wide chest x-ray survey has developed over the past four years, it has become a concentrated attack on tuberculosis with positive value in the discovery and consequent control of the disease. Led by the practicing physician and the medical society, the mass chest x-ray survey is based on an amalgamation of the efforts of all the resources, skills, interests, and knowledge of an entire community.

The United States Public Health Service, participating in 14 community-wide chest x-ray surveys, has aided in the small-film screening of nearly 5½ million persons through the use of the photofluorograph. Recognizing that the x-ray had limitations as a diagnostic tool for tuberculosis, the Public Health Service concurrently set up two research facilities in an effort to minimize these limitations as well as to provide knowledge for sharpening and refining mass survey procedures. One of these, the Central Cooperative Clinic in Philadelphia, is following the health histories of tuberculosis suspects found in industrial surveys. The other study, located in Muscogee County, Georgia, is investigating the occurrence and spread of tuberculosis within a community following an intensive case finding program. Information from these studies, it is hoped, should clarify many problems, including the relationship of exogenous and endogenous infections.

In community-wide surveys, the goal of screening operations is usually set at 80 per cent of the eligible population 15 years of age and over. In actual operations, total small films taken range between 55 per cent of this eligible population (in St. Paul, Minn.) and 93 per cent (in Salt Lake City). Generally, the two sexes respond more or less equally. Response by race, however, varied from one city to another, with younger age groups usually responding better than older age groups. Identification of previously unknown cases of active tuberculosis represents one of the major contributions of the community wide survey, and it is believed that 85 per cent to 90 per cent of the active cases are known to the health department.

In the second stage of survey operations, those whose small-film findings are suggestive of tuberculosis are recalled for confirmatory 14" x 17" films. Correlations between positive large film findings and economic status in Seattle and Cleveland appear to confirm the commonly held notion that tuberculosis prevails to a greater extent at the lower economic levels. While in most surveys referral to private phy-

sicians or public clinics occurs immediately after the confirmatory large films are taken and read, in some cities (Denver, for example) further diagnostic study is undertaken before a referral is made.

In the Boston survey of 1949, special procedures were instituted for the follow-up of thoracic neoplasms as well as identification of cases of cardiovascular abnormalities. A direct correlation was found between the prevalence of cancer and age, with a higher frequency of neoplasms occurring among men than among women. While readings of cardiac abnormalities have appeared in 2 to 10 x-rays of every 1,000 taken in community-wide surveys, there is disagreement regarding the value of the x-ray in finding heart disease that can be benefited medically. It is hoped that further observations from the Boston survey will help to resolve these divergent views.

Where physicians have conducted complete follow-up on all referred cases and have reported to the survey organization, the structure of knowledge about tuberculosis is being built up and control programs are being modified to conform with the knowledge gained. With the survey's stimulus and the increase of public interest in and support for tuberculosis control, improvements in the program have been made possible in many places. In the future, we can expect that the mass approach to tuberculosis case finding will make even greater contributions to the fight against tuberculosis in the United States. 14 references. 7 figures.

INTEGRATION WITH ALLIED SPECIALTIES

164. *N-Allyl Normorphine in the Treatment of Morphine or Demerol Narcosis.* JAMES E. ECKENHOFF, JOHN D. ELDER, JR. AND BENTON D. KING, Philadelphia, Pa. Am. J. M. Sc. *223*:191-97, February 1952.

N-allyl normorphine has been used in 27 patients who have been given large doses of morphine (15 to 90 mg.), demerol (150 to 600 mg.) or who have had cyclopropane, ether or pentothal as the primary anesthetic agent. Where respiration was depressed with either morphine or demerol, 5 or 10 mg. of n-allyl normorphine intravenously, resulted in a two or three fold increase in respiratory rate and a 200 to 300 per cent increase in respiratory minute volume. The peak increase in minute volume was reached within 2 minutes, then declined, but remained well above the depressed level. Pulse rate in the depressed individual declined after n-allyl normorphine. If the patient was hypotensive due to the narcosis, n-allyl normorphine uniformly caused a return toward the normal pressure, the peak rise accruing 5 to 10 minutes after injection. In normotensive patients there was no significant effect on blood pressure. A slight analeptic action was produced by the drug. N-allyl normorphine had no effect on depression produced by cyclopropane, pentothal or ether.

N-allyl normorphine was administered intravenously (5 or 10 mg.) to 5 normal male volunteers. Respiratory rate was not remarkably affected. Respiratory minute volume was uniformly diminished (an average of 35 per cent), the depression reaching maximum in 5 to 10 minutes and persisting for 40 or more minutes. Pulse rate and blood pressure were unchanged, except in one individual whose pressure fell from

120/70 to 70/40. When tilted to 60 degrees, 2 subjects fainted—one in 9 and the other in 10 minutes. All patients complained of a "tired feeling," and all developed a pallor similar to that observed when morphine is administered intravenously to normal subjects.

The therapeutic use of the drug in 2 patients accidentally depressed with overdoses of morphine are described. In both instances the antagonistic effect to the opiate was excellent. 14 references. 1 figure. 1 table.—*Author's abstract.*

MUSCULOSKELETAL

165. *The Effect of Cortone and ACTH on the Histopathologic Lesions of Rheumatoid Arthritis.* BERNARD M. NORCROSS; MAXWELL LOCKIE; ANTHONY G. CONSTANTINE; JOHN R. TALBOTT, AND ROBERT H. STEIN, Buffalo, N. Y. Ann. Int. Med. *36*:751-64, March 1952.

In the 30 cases of rheumatoid arthritis studied, 27 were cases of peripheral arthritis, 2 were cases of rheumatoid spondylitis, and 1 a case of rheumatoid arthritis with psoriasis. Muscle biopsy specimens were obtained in these cases before beginning therapy with Cortone or ACTH, and at various periods during treatment, if the initial specimens showed significant changes. Both inflammatory nodules and degenerative changes of muscle fibers were studied in these biopsy specimens. Such lesions were found in 18 of the 30 patients, but were considered to be of a significant degree (classed as Grade II or III) to evaluate the effect of the treatment in only 11 patients. In 8 of these 11 patients definite improvement in both types of lesions was found to result from treatment with Cortone or ACTH. In 5 patients the inflammatory nodules disappeared, and in the other 3 patients, there was definite improvement. Improvement in the degenerative lesions was noted in all patients, but was of a lesser degree than the improvement in the inflammatory lesions. Subcutaneous rheumatic nodules were carefully studied in 12 patients; in 3 of these patients nodules of the same stage of development were removed before and during the course of treatment. In all cases, there was considerable diminution in the size of the nodules during treatment and they became less painful and appeared less inflammatory; some of the smaller nodules disappeared entirely. In 2 of the 3 patients in whom the nodules were studied histologically, there was a definite histologic change, including diminution of round cell infiltration. In the 2 patients in whom biopsy studies of the synovial membrane were made, there was marked reduction of the inflammatory lesions. No delay in healing of the multiple incisions made to obtain biopsy specimens was noted in this group of patients during treatment with Cortone or ACTH. 13 references. 10 figures.

CARDIOVASCULAR

166. *Effect of ACTH and Cortisone on Rheumatic Carditis. Observations of Eighteen Cases.* A. G. KUTTNER, J. S. BALDWIN, CURRIER MC EWEN, J. J. BUNIM, MORRIS ZIFF AND D. K. FORD, New York, N. Y. J.A.M.A. *148*:628-39, February 23, 1952.

Eighteen patients with unequivocal signs of rheumatic carditis were treated with

ACTH, or cortisone, or by ACTH followed by cortisone. These hormones usually produced a prompt improvement in the general condition of the patients: the temperature decreased, toxicity disappeared, joint manifestations subsided, appetite improved. Various abnormal laboratory findings (the C-reactive protein, ESR and gamma globulin) tended to become normal. These effects were most striking in acutely ill, febrile patients. They were obtained both with ACTH and cortisone, but usually occurred more quickly with ACTH.

The immediate effect of ACTH and cortisone on the various manifestations of carditis: pericarditis, myocarditis as indicated by a decrease in heart size, subsidences of congestive failure, disappearance of gallop rhythm and tachycardia, or decrease in the lability of cardiac rate, and endocarditis as judged by changes in murmurs.

In this group of patients the manifestations of carditis which responded most favorably were pericarditis and congestive failure precipitated by severe myocarditis. Although the data suggested that pericarditis was favorably affected, the duration of this manifestation is known to be variable, and it was difficult to be sure that the disappearance of pericarditis was definitely accelerated.

The beneficial effect on congestive failure was more striking. Six of the 18 patients showed this manifestation; 5 responded, 4 to hormone therapy alone. The fifth patient, a $2\frac{1}{2}$ year old child, critically ill, recovered on ACTH, digitalis, and mercuhydrin. The sixth patient in whom signs of congestive failure persisted received relatively small doses. This girl had a severely damaged heart and had had continuous rheumatic activity for nine months.

In the absence of pericardial effusion no significant decrease in heart size was observed. No striking changes in murmurs were noted. In most patients gallop rhythm disappeared, and the quality of the heart sounds improved. Tachycardia in the absence of fever and lability of the cardiac rate were the two signs of carditis which responded least.

Twelve patients were treated during their first rheumatic attack at varying intervals from the time of onset. Eight received a single course of therapy one to seven weeks following the first appearance of their rheumatic manifestations. Active carditis subsided during treatment in 4 of these eight children, but in the other 4 clinical evidence of rheumatic activity persisted for six to nine weeks after cessation of therapy.

The remaining 4 of these 12 patients were treated several months after onset; 3 received two courses of therapy. However, carditis did not subside until five to seven weeks after the end of the second course. In the fourth child the rheumatic process was still considered to be active after three courses.

Observations made two to nine months after complete cessation of therapy showed that all 12 patients had murmurs indicative of organic heart disease. Cardiac enlargement was definite in all but 1 case.

Six patients with antecedent rheumatic heart disease were treated during recurrent attacks. Therapy was instituted at intervals varying from 1 to 38 weeks from onset. In 2 patients, signs of activity disappeared within three weeks after therapy was begun, but relapse occurred in both within ten days after the hormones were withdrawn. In 2 patients, active carditis persisted for nine and 16 weeks, respectively, and in 2 patients for longer than 16 weeks.

Signs of hyperadrenalism were observed frequently but in every instance disappeared gradually after cessation of therapy.

In this group of patients, it was not clear whether the use of ACTH or cortisone significantly decreased the duration of active carditis or influenced cardiac damage. Further studies with a greater number of patients and prolonged periods of observations are necessary. 4 references. 1 figure. 4 tables.—*Author's abstract.*

ALLERGY

167. *Cardiovascular Allergy Due to Penicillin, Sulfadiazine, and Bacterial Sensitization. Results of Treatment with Cortisone and ACTH.* JOSEPH HARKAVY, New York, N. Y. J. Allergy 23:104-22, March 1952.

This report deals with 4 patients, each of whom had a personal history of allergy, characterized by vasomotor rhinitis, chronic sinusitis with and without polyps, and recurrent episodes of asthma or cough.

The first 2 cases, females aged 50 and 43, developed a serum sickness-type of response following the administration of penicillin. The penicillin in the first case was given both intramuscularly and by aerosol prior to and after polypectomy for chronic polypoid sinusitis, in the second case, because of an acute pansinusitis. Ten to 14 days later, each of these patients developed fever of 101 and 102 F., migratory pulmonary infiltrations with serous effusions, generalized vesicular and necrotizing purpura, polyarthritis with eosinophilia of 35 to 40 per cent. Concurrently, there appeared tachycardia with electrocardiographic changes indicative of myocardial damage, such as right bundle branch block, etc. Renal involvement with terminal azotemia was noted in 1 of the 2 cases. Therapy with cortisone and ACTH was initiated three to four weeks after onset of symptoms. Both of these cases developed buttock abscesses containing *Staphylococcus aureus* in the course of treatment necessitating an increase in the dosage of the cortisone or ACTH, respectively. Although temporary remissions in the purpura, polyarthritis, temperature as well as complete and permanent disappearance of the pulmonary infiltration occurred, as a result of hormonal therapy, both patients went on to exitus despite intensive treatment.

Autopsy of case one, aged 50, showed: (1) allergic granulomatosis of the aorta, generalized necrotizing arteritis and focal fibrosis, diffuse myocardial fibrosis; (2) partial atelectasis, edema, and congestion of the lungs; (3) ulceration and erosions of the entire gastro-intestinal tract, acute gastric ulcers with passive bleeding; (4) acute hepatic necrosis; (5) infected deep ulcerations of skin over sacrum, buttocks, and thighs. Biopsies of the purpuric lesions in case two showed changes compatible with allergic granulomatosis. There was also noted marked arteritis and muscle atrophy but no evidence of periarteritis nodosa.

Diagnosis. Hyperergic vascular disease due to penicillin sensitization. Case three, male aged 45, received sulfadiazine for a respiratory infection in 1945. Several days later he developed pruritus, progressive malaise, general weakness followed by gradual enlargement of the abdomen. In 1947 he was found to have an enlarged liver and spleen with WBC of 30.000 and 60 per cent eosinophiles; this progressed. In January

1949, in addition to the abdominal findings, examination disclosed infected sinuses, pulmonary infiltrations with wheezing respirations, and increase in the size of the heart with marked electrocardiographic changes indicative of myocardial damage. Sinus washings yielded a "Taylor" *Pneumococcus* on culture. X-ray of the lungs showed pneumonic lesion at left base. Intramuscular penicillin therapy cleared the sinus infection; aerosol penicillin, however, precipitated an asthmatic crisis. In October 1949 he developed a severe attack of left upper quadrant pain with precipitous augmentation in the size of the spleen. A diagnosis of splenic infarction was made on the basis of hyperergic vascular disease. The spleen was removed and biopsies of liver and lymph node showed the following: splenomegaly (weight 1100 Gm.) with stenosing endophlebitis and focal obliterating arteritis as well as periarteriolar sclerofibrosis; diffuse eosinophilic infiltration of the splenic pulp with occasional myelocytes and megakaryocytes, increase of fibrillar reticulum together with multiple anemic infarcts. The liver showed periportal eosinophilic infiltration; An excised lymph node revealed eosinophilic lymphadenitis with focal fibrosis.

Following splenectomy his white cell count rose to 44.000 and 75 per cent eosinophiles. He also developed congestive heart failure, which was treated with salt free diet, mercurials, etc. Cautious administration of ACTH reduced the eosinophiles to 5 per cent. The general condition, however, grew worse. He developed signs of cerebral involvement and died. A diagnosis of diffused hyperergic vascular disease with eosinophilic myocarditis probably due to sulfonamide sensitivity and or bacterial allergy was entertained.

The fourth case, female aged 17, was seen first in 1948 because of fever to 101 to 102 F, cough, night sweats, tachycardia, and chronic sinus involvement. X-ray examination of the lungs revealed migratory pulmonary infiltrations of the Loeffler type. These fluctuated from interstitial infiltrations to massive involvement of one lobe or another. At one time atelectasis of one lobe with a shift of the heart to the side of the collapsed lung was noted. Early electrocardiograms showed tachycardia. WBC 29.000 with 29 per cent eosinophiles. Sensitivity was manifested to penicillin, aureomycin, and chloromycetin characterized by purpura. These drugs were, therefore, discontinued. The pulmonary infiltrations with occasional remissions continued to appear. She developed anemia, necessitating transfusions, and signs of cardiac insufficiency despite salt free diet, etc. ECG showed right bundle branch block of the Wilson type. At this point (October 1949) cortisone became available and was administered in 150 mg. doses, daily. This caused complete disappearance of the pulmonary infiltrations and fever which had prevailed on and off for 18 months. Peripheral neuritis which she had developed also disappeared. Cortisone was stopped December 27, 1949. Four weeks later the pulmonary lesions recurred. She was given a second course of cortisone therapy with complete resolution of the lung infiltrations. On the thirty-sixth day of treatment after injection of 50 mg. of cortisone, she sustained an anaphylactic-like shock with wheezing respiration. This lasted for several minutes with recovery in 48 hours. The cause was found to be sensitivity to the cortisone vehicle. Following this episode the lungs have remained clear. Except for an occasional asthmatic attack, which is readily controlled by oral cortisone, the patient is free of symptoms. The

abnormal electrocardiogram however, indicating right bundle branch block has persisted to date.

The precise etiology in this case of vascular allergy could not be established, although respiratory infection was suspected.

This group of cases indicate that ACTH and cortisone will influence reversible processes such as pulmonary infiltrations, purpura, polyarthritis, etc. They have no effect on irreversible cardiovascular changes. The possibility of development of intercurrent infection in prolonged hormonal therapy must always be borne in mind. 33 references. 9 figures.—*Author's abstract.*

INFECTIOUS DISEASE

168. *Klebsiella Pneumoniae Meningitis.* ALVIN J. THOMPSON, EDWARD B. WILLIAMS, JR. EDWIN D. WILLIAMS AND JOHN M. ANDERSON, St. Louis, Mo. Arch. Int. Med. *89*:405-20, March 1952.

In the case reported, the patient, a woman 61 years of age, had symptoms of involvement of the left lung. The sputum was "stringy," and mucoid and *Klebsiella pneumoniae* (*Friedländer's bacillus*) was isolated in cultures. Symptoms of meningitis developed, and the same organism was isolated by culture from the spinal fluid. *In vitro* tests showed this organism to be sensitive to aureomycin and streptomycin, but not to penicillin. All three antibiotics were used in treatment; penicillin had been given intrathecally, but streptomycin was substituted for intrathecal treatment.

The patient made a good recovery. She had been found to be a diabetic and was given insulin; but as the infection subsided, it was found that the diabetes could be controlled by diet alone. In a review of the literature it was found that meningitis due to the *Friedländer bacillus* is of rare occurrence; 118 cases were collected from the published reports. The disease was usually fatal before the sulfonamides and the antibiotics became available. Streptomycin, aureomycin, chloramphenicol, and terramycin have been found to have an antibacterial action against most strains of *K. pneumoniae*, but which antibiotic will prove most effective in treatment has not yet been determined. 26 references. 1 figure. 3 tables.

GENITOURINARY DISEASE

169. *Treatment of Syphilis with Aureomycin and Chloromycetin.* S. R. TAGGART, M. J. ROMANSKY, AND G. S. LANDMAN, Washington, D. C. Am. J. Syph. Gonor. & Ven. Dis. *36*:174-78, March 1952.

This paper is a final report on a 12 to 15 month follow-up of two groups of approximately 100 patients each treated with orally administered aureomycin and chloromycetin.

It was observed that the spirochetes disappear from the surface lesions of early

syphilis in 22 to 30 hours after the administration of aureomycin or chloromycetin in the range of 240 to 30 mg. per Kg. per day.

The accumulative percentage retreated and per cent attaining sero-negativity at 12 to 15 months with a dosage of 60 mg. per Kg. per day, six to eight days, was comparable to that reported following the use of 300,000 units or more of penicillin a day for seven and one-half to ten days. Retreatment rates were high in all dosage ranges used (30 to 240 mg. per Kg. per day), if the time of treatment was fours days or less. All patients treated for only one day had clinical relapse.

Healing of primary, secondary and late syphilides was as rapid as that following penicillin treatment.

In the groups studied, 12 patients had gonorrheal urethritis, chancroid, or granuloma inguinale in combination with syphilis, and on the treatment schedules used for syphilis, these associated diseases were cured.

The status of 22 infants born of syphilitic women treated with the two antibiotics were reported with only two failures. These treatment failures occurred on dosages of 30 mg. per Kg. per day for four days, one on each antibiotic.

Reactions from treatment with the two antibiotics were related mainly to the gastro-intestinal tract and were somewhat more severe with aureomycin than from chloromycetin treatment. Vitamin B deficiency was noted in 7 patients—5 with chloromycetin and 2 with aureomycin.

The authors conclude that: aureomycin and chloromycetin, administered orally, are shown to be therapeutically active in syphilis. The present study indicates that the results obtained in the various stages of syphilis, following the administration of 60 mg. per Kg. per day for eight days, are comparable to those following the use of intramuscular penicillin over a comparable period of time.

Aureomycin and chloromycetin may be used for the treatment of syphilitic patients for whom oral administration is to be preferred to parenteral use.

Aureomycin and chloromycetin may be used in the treatment of patients with syphilis who are allergic to penicillin.

Aureomycin and chloromycetin are effective in the treatment of chancroid and granuloma inguinale, against which penicillin is ineffective. The use of aureomycin and chloromycetin for the treatment of these two diseases makes additional medication with penicillin unnecessary when these two diseases are associated with syphilis in the patient under treatment. 3 references. 2 tables.—*Author's abstract.*

DERMATOLOGY

170. *Inhibition of Melanin Formation by Chemical Agents.* CLEVELAND R. DENTON, AARON BUNSEN LERNER, AND THOMAS B. FITZPATRICK, Ann Arbor, Mich. J. Invest. Dermat. *18*:119-35, February 1952.

It has been shown through clinical trial, animal experiments, and *in vitro* studies that hydroquinone and p-hydroxypropiophenone are effective inhibitors of melanin formation. Monobenzylether of hydroquinone was the most effective inhibitor of

melanin formation clinically, although it had no effect on melanin formation *in vitro.* These compounds when administered orally to colored guinea pigs produce depigmentation. The parenteral administration of hydroquinone and p-hydroxy-propiophenone to black mice also produces depigmentation. Monobenzylether of hydroquinone in 10 to 33 per cent concentration in lotions and ointments is an effective agent clinically for treatment of hyperpigmentation. 23 references. 12 figures. 3 tables.—*Author's abstract.*

171. *Crossed Fixed Drug Eruption from Two Antibiotics.* ASTON L. WELSH, Cincinnati, O. Arch. Dermat. & Syph. 65:232, February 1952.

Fixed drug eruption from aureomycin was reported previously.

Terramycin, ingested on two occasions between periods of ingestion of aureomycin and preceding fixed eruption produced by the latter drug, produced no untoward reaction.

After eruption appeared from aureomycin, terramycin was administered to complete treatment of an upper respiratory infection. Lesions of the fixed eruption from aureomycin became more marked and persisted much longer than earlier episodes of eruption, in the same sites produced by aureomycin alone.

Six months later, terramycin (one dose) again taken for another attack of upper respiratory infection, was followed by pruritus and eruption. Pigmentation at sites of the fixed eruption from aureomycin became more pronounced.

This same series of events occurred a second time after a single dose of terramycin taken to determine positively that this drug was capable of producing the cross fixed drug eruption already described.

Observation suggests some common sensitizing factor in aureomycin and terramycin. 1 reference.—*Author's abstract.*

GASTROINTESTINAL

172. *Studies on the Agent of Infectious Hepatitis. II. The Effect of Skin Tests for Infectious Hepatitis on the Incidence of the Disease in a Closed Institution.* MILES E. DRAKE, CHARLES WARD, JOSEPH STOKES, JR., WERNER HENLE, GEORGE C. MEDAIRY, FRANCOISE MANGOLD, AND GERTRUDE HENLE, Philadelphia, Pa. J. Exper. Med. 95:231-39, March 1952.

In an effort to determine the meaning of positive and negative skin tests for infectious hepatitis in terms of immunity or susceptibility, about one quarter of the inmates with negative histories of jaundice in a closed mental institution were injected intradermally with antigen (and corresponding control material) at the onset of an outbreak of the disease. Of these, 144 gave a positive reaction, and 176 were found negative. Although the epidemic continued for a period of one year, few cases of jaundice occurred in the skin-tested group. Among the 825 individuals not skin-

tested, 112 cases of jaundice were recorded or an incidence of 13.6 per cent, whereas only 5 cases were noted among 320 previously skin-tested, or an incidence of 1.6 per cent. One of the latter cases had given a positive reaction. In two of the cottages, housing boys and girls of the youngest age group (4-14 years), respectively, the incidence of jaundice was highest. Yet the same relative difference in the incidence of jaundice between skin-tested (mostly negative) and nonskin-tested individuals was apparent. Examination of the institutional records of these children as to length of stay at the institution failed to reveal an inadvertent selection, thus excluding the possibility that the skin tested group contained larger numbers of children immunized by exposure to IH during an outbreak which had occurred five to six years previously. These data suggest that the skin test antigen had altered the susceptibility. Possible explanations for this observation have been discussed. 12 references. 5 tables.—*Author's abstract.*

METABOLIC & ENDOCRINE DISORDERS

173. *Mycosis of Cervical Spinal Cord Following Intrathecal Penicillin Therapy. Report of a Case of Simulating Cord Tumor.* ROBERT E. WYBEL, Columbus, Ohio. Arch. Path. 53:167-73, February 1952.

Mycotic infections occurring during antibiotic therapy and reports of experimental evidence that the growth of fungi is enhanced by antibiotic agents have appeared in the recent literature. The case described here is an example of a fatal mycotic granuloma of the cervical spinal cord that followed intrathecal penicillin administration.

A 40 year old housewife developed chills, fever, headache and a stiff neck. She was admitted to University Hospital where the characteristic physical findings of acute meningitis were observed and Type III pneumococci were cultured from the spinal fluid. Intramuscular and intrathecal penicillin and oral sulfadiazine were given and the patient made a very satisfactory recovery.

However, following discharge, she gradually developed weakness, tremors of the hands and progressive difficulty in walking. Within three years after her meningitis she had developed a complete quadriplegia and a diagnosis of cord tumor was made. The patient died of respiratory paralysis following surgical exploration of the spinal cord.

Autopsy showed an inflammatory mass surrounding the cervical spinal cord. Histologically the mass was a pseudotuberculous granuloma composed of many tubercles with purulent centers containing hyphae and spores. The organism was identified morphologically as *Aspergillus.*

The lesion appeared to be of an age compatible with origin at the time of intrathecal penicillin therapy. The fungi may have been introduced as contaminants during lumbar puncture. The intrathecal penicillin and other antibiotics administered to the patient for treatment of her meningitis probably played a role in the development of the mycotic granuloma.

Examples of overgrowth of fungi in the intestine, mouth, vagina and in the lungs during antibiotic therapy, as well as the occurrence of mycotic infections and generalized dissemination of fungi during antibiotic therapy are cited from the literature and the experimental evidence of enhancement of the growth of fungi by antibiotic agents is discussed. 13 references.—*Author's abstract.*

BASIC SCIENCES

174. *A Comparison of the Renal Effects of Polymyxin A, Aerosporin and a Mercurial Diuretic.* AMOS E. LIGHT, JOHN A. TORNABEN AND EDWIN J. DE BEER, Tuckahoe, N. Y. Antibiotics and Chemotherapy *2*:63-71, February 1952.

The effectiveness of 'Aerosporin'[1] against certain gram negative organisms has been well established, but there has been occasional reluctance to use the drug because of possible nephrotoxic effects. This study was undertaken to investigate the nature and the degree of the action of 'Aerosporin' on the kidney. Rats were used as experimental animals.

At high dosage levels the drug produced a diuresis. The 24 hour protein output was not increased to a like degree and consequently the urinary protein concentration actually diminished. Histologic examination of the kidney revealed a distention of the tubules. The glomerular tufts appeared to be undamaged. The changes observed were not permanent for a discontinuance of the antibiotic resulted in a prompt return to normal conditions.

Polymyxin A produced effects which were more serious and prolonged but here also the abnormalities disappeared when dosing was discontinued.

There were many points of resemblance between the polymyxins and mercurial diuretics.

It was concluded that the action of 'Aerosporin' on the rat's kidney was chiefly diuretic in nature and that the effects were transient in character. 15 references. 2 figures. 2 tables.—*Author's abstract.*

175. *The Cellular Transfer in Humans of Delayed Cutaneous Reactivity to Hemolytic Streptococci.* H. SHERWOOD LAWRENCE, New York, N. Y. J. Immunol. *68*:159-78, February 1952.

It was shown by Chase that specific cutaneous hypersensitivity of the delayed type to tuberculin can be transferred passively to unsensitized guinea pigs by the injection of leucocytes isolated from the peritoneal exudates, spleen or lymph nodes of sensitized guinea pigs.

Following this lead we were able to demonstrate similar passive transfer of specific local and generalized cutaneous hypersensitivity of the delayed type to tuberculin in man. This was accomplished by the intradermal injection of viable leucocytes isolated

from the peripheral blood of tuberculin sensitive, non-tuberculous humans. The findings in man were essentially the same as those previously reported by Chase in the guinea pig.

Using this observation as a model of the cellular transfer of the delayed type of bacterial hypersensitivity, the method was adapted to the study of the delayed cutaneous reactions to the beta hemolytic streptococcus and its components in man.

In a series of 472 adult patients without apparent streptococcal disease or its complications, the incidence of positive delayed cutaneous reactions was as follows:

a) To intact Streptococcal cells—39 per cent of 67 patients.
b) To Streptokinase-Streptodornase (SK-SD)—69 per cent of 112 patients.
c) To Streptococcal M Substance—36 per cent of 293 patients.
d) Of the total 472 patients, the overall incidence of positive reactions to the streptococcal materials was 45 per cent.

It has been possible to transfer passively, in 14 consecutive instances, generalized cutaneous streptococcal reactivity of the delayed tuberculin type to streptococcus-negative human recipients by means of viable leucocytes obtained from streptococcus-positive human donors.

This was accomplished with intact streptococcal cells as the test material in 4 instances, with SK-SD as the test material in 6 instances, and with M Substance as the test material in 3 instances. In one instance it was possible to induce delayed cutaneous reactions to intact streptococcal sells, SK-SD and M Substance simultaneously in the same negative recipient.

Delayed cutaneous reactions did not develop in the streptococcus-negative recipient when the transferred leucocytes were obtained from streptococcus-negative donors.

The endowment of the negative recipient with the capacity to develop generalized delayed cutaneous reactivity to streptococcal materials, following leucocytic transfer, is a transient phenomenon.

The results of leucocytic transfer of delayed cutaneous reactivity to hemolytic streptococcal materials in humans generally appear to parallel those reported in humans using tuberculin as the test substance. 26 references. 5 figures. 7 tables.—*Author's abstract.*

book reviews

METABOLIC INTERRELATIONS: *Transactions of the Third Conference of the Josiah Macy Jr. Foundation, January 8-9, 1951.* Edited by Edward G. Reifenstein. Jr. 294 pages. Josiah Macy Jr. Foundation, New York City, 1951. Price $4.00.

This book follows the format of the Josiah Macy Jr. Foundation. It is an edited transcription of a conference on bone metabolism attended by many of the foremost investigators in the field. Some of the experimental problems described are: resorption of bone; osteogenesis; the effect of altering serum calcium concentration; the effect of beryllium salts on calcification; the effects of pituitary factors and thyroxin on skeletal morphogenesis; and observations on the role of phosphotase.

Hastings reported on attempts to produce tetany in a recipient dog by removal of

serum calcium from the donor dog. Although the calcium was reduced to about 7 mgm. per cent, tetany could not be produced, underscoring the dynamic state of the calcium depots. The role of phosphatase in calcification is further elucidated by Neuman et al, who have attacked this problem with a new approach; that is, that ester phosphate may actually be inhibitory for calcification and that phosphatase may act as the trigger to release such inhibition. Experiments proved that there is an interference with the absorption of ester phosphate by phosphatase so that a process which is inhibitory for growth of the mineral crystals is eliminated. Further experimental evidence for the vital hormone influence on skeletal morphogenesis is presented by Asling and his colleagues. In normal rats, growth hormone neither accelerates or retards epiphyseal fusion. From this observation further studies on hypophysectomized and thyroidectomized rats with or without growth hormone or thyroid hormone are reported.

The discussions following each presentation are particularly invaluable. Not only do they add to the interpretation to the foregoing essays, but in many cases related problems are considered, posing new concepts or new lines of experimental approach.

The average practitioner will find most of the discussion too technical and based upon a fundamental knowledge in chemistry and histology. However this is an invaluable reference for a physician working in this field. Some of the presentations lack sufficient clarity because of the lack of figures and diagrams that undoubtedly accompanied the original presentation.—*Leonard Meiseles.*

FACTORS REGULATING BLOOD PRESSURE — *Transactions of the Fifth Conference, February 15-16, 1951.* Edited by Benjamin W. Zweifach and Ephraim Shorr, Cornell University Medical College, New York, 1951, Josiah Macy Jr. Foundation, 238 pages. Price $3.75.

The present volume represents the fifth and final Josiah Macy Conference on Factors Regulating Blood Pressure. As in the other volumes it is the discussion and interplay of ideas perhaps more than the new data which make this series so valuable.

Of interest is the main theme of the present conference compared to the first. In the latter, experimental hypertension induced by renal artery compression permeated the discussion while only two out of eleven papers concerned with this subject are noted in the present report. The other discussions center about the genesis of arteriosclerosis with emphasis on lipid metabolism. Here the main stimulus has undoubtedly come from research utilizing the ultra-centrifugal differentiation and clinical correlation of the lipoprotein complexes in plasma.

The discussion raises but does not answer such fundamental questions as the relation of exogenous to endogenous cholesterol in atherogenesis; the role of serum lipid changes in lipid deposition as compared to local physical factors in the artery; the mode of entry and deposition of lipid in the arterial intima and, of most significance, whether the abnormal serum patterns represent a "primary" disorder or merely reflect some more profound metabolic imbalance. The amelioration of experimental renal hypertension with anti-renin is reported and the problem of producing human anti-renin discussed.—*William Ruberman.*